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PATHOLOGY

A Periodical Devoted to General and Experimental Pathology

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TABLE OF CONTENTS

VOLUME 67 MARCH 1959 NUMBER 3

ORIGINAL ARTICLES

PAGE

Studies on Ferrodynamics
Joseph P. Wack, Ph.D., and John P. Wyatt, M.D., St. Louis
I. Gastrointestinal Absorption of Fe^{59} in the Rat Under Differing Dietary States 237
II. The Uptake and Distribution of Labeled Fe^{59} in Experimental Infusional Siderosis 243

Gastric Mucormycosis in a Newborn Infant
Robert R. Gatling, M.D., Roanoke, Va. 249

Irradiation Injury to the Aorta and the Lung
Elizabeth Thomas, M.B., B.S., and Wiley D. Forbus, M.D., Durham, N. C. 256

The Fate of Equine-to-Canine Arterial Heterografts
Walter S. Henly, M.D.; E. Stanley Crawford, M.D.; Michael E. De Bakey, M.D., and Béla Halpert, M.D., Houston, Texas 264

Immunochemical Demonstration of Fibrin in the Generalized Shwartzman Reaction
Donald G. McKay, M.D., Brookline, Mass.; David Gitlin, M.D., and John M. Craig, M.D., Boston 270

Progressive Cellular Alterations of Lymph Nodes
Richard D. Moore, M.D.; George D. Sorenson, M.D., and Melvin D. Schoenberg, Ph.D., Cleveland 274

Histochemical Study of Aminopeptidase in Metaplasia and Carcinoma of the Stomach
Lee W. Wattenberg, M.D., Minneapolis 281

Changes in Desquamated Cells in Carcinogenesis
James W. Reagan, M.D., and W. Budd Wentz, M.D., Cleveland 287

Histochemistry of Breast Diseases
Herbert Fanger, M.D., and Barbara E. Barker, M.S., Providence, R. I. ... 293

Experimental Cyanide Encephalopathy
Seymour Levine, M.D., and Walter Stypulkowski, B.S., Jersey City, N. J. 306

Further Histochemical Studies of Esterases by 5-Bromoindoxyl Acetate
Bjarne Pearson, M.D., M.S., and Fred Grose, B.S., Detroit 324

Intestinal Pneumatosis
Laurence P. Skendzel, M.D., Indianapolis 333

Fatal Cases of Acute Sicklemia
Hsiang Len Tseng, M.D., Baltimore 339

OBITUARIES

Nathan Chandler Foot, M.D. 347

REGULAR DEPARTMENTS

News and Comment 350

Books 353

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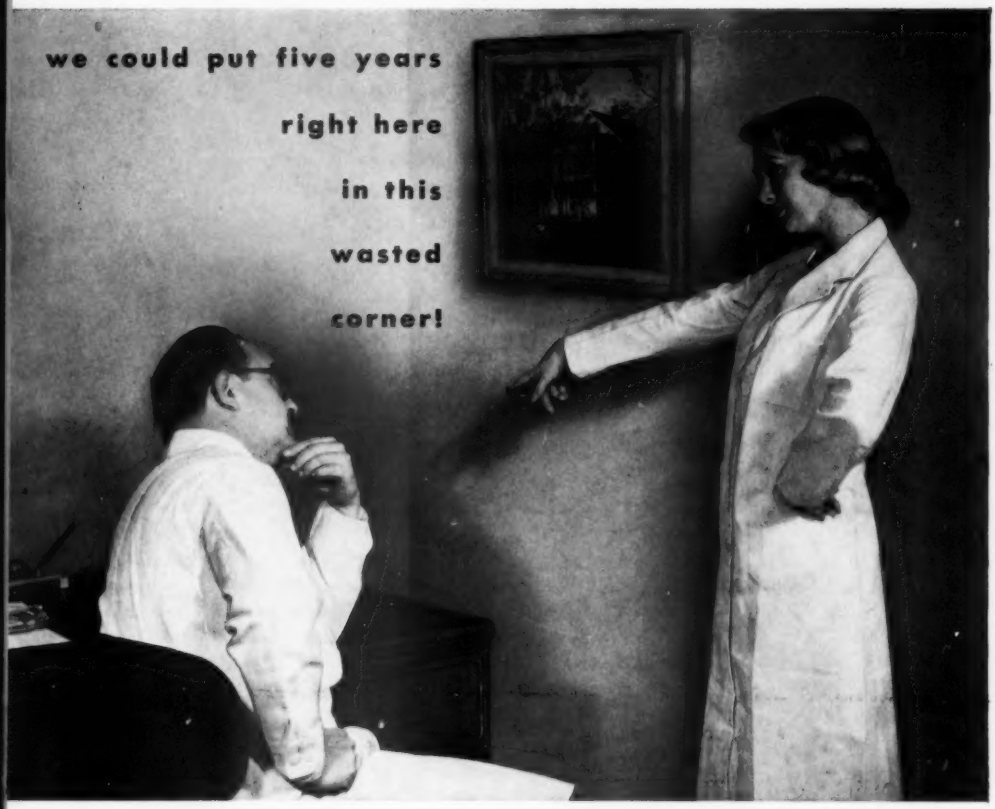

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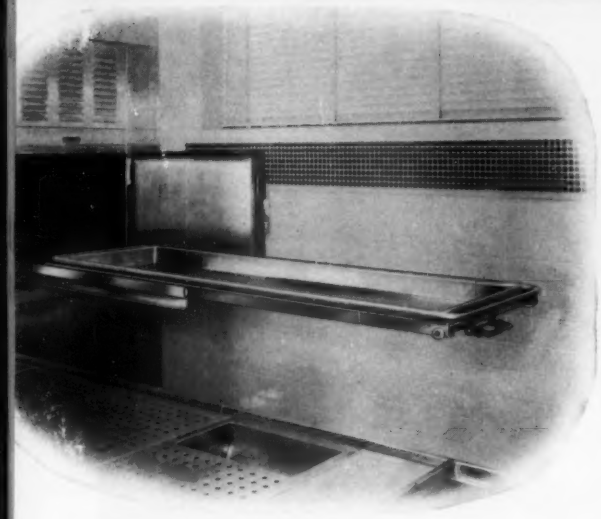

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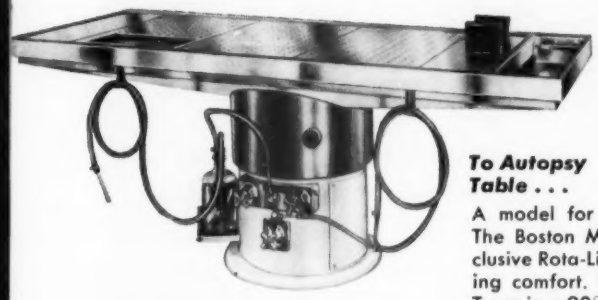

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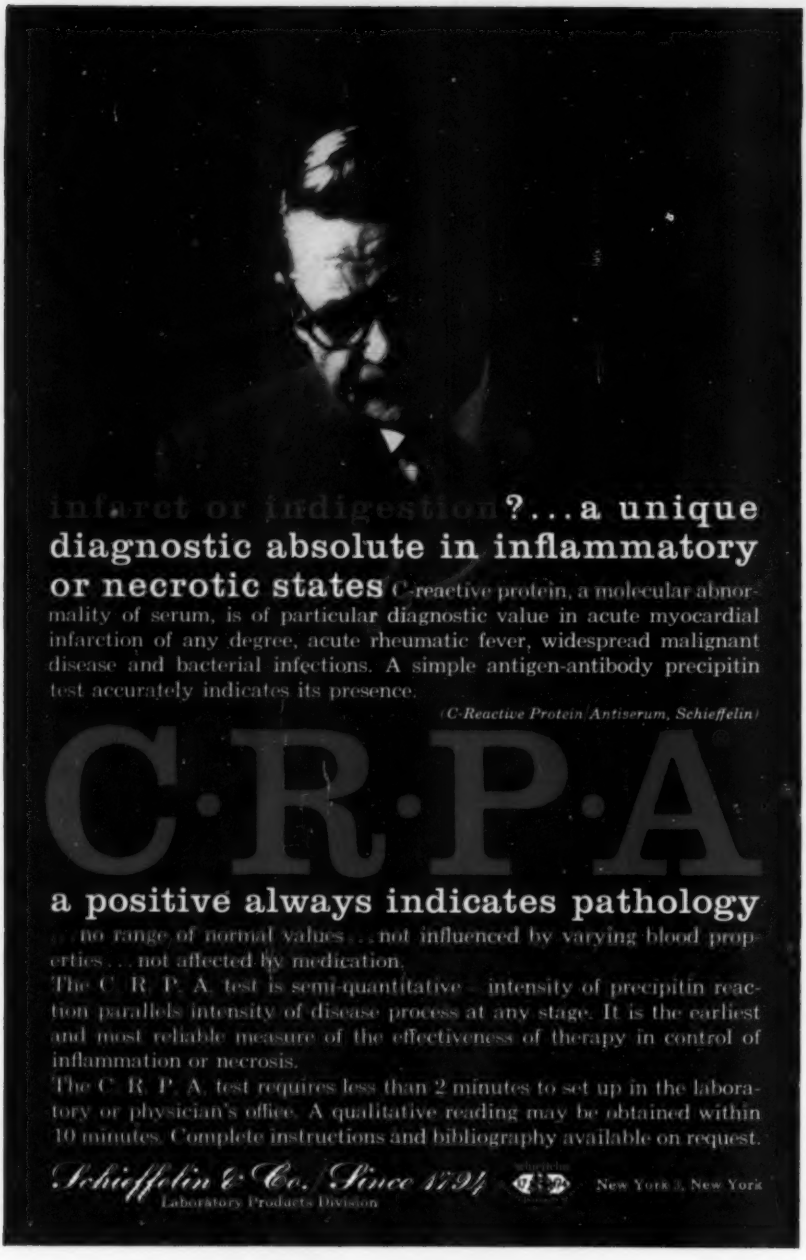
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Studies on Ferrodynamics

I. Gastrointestinal Absorption of Fe^{59} in the Rat Under Differing Dietary States

JOSEPH P. WACK, Ph.D., and JOHN P. WYATT, M.D., St. Louis

Under normal conditions, the absorption of iron in the ferrous form is sharply confined to the duodenal region, but, under certain pathologic conditions, the iron may be absorbed throughout the length of the gastrointestinal tract.[1-4] This duodenal absorption zone is probably due to the chemical milieu of the pars duodenum favoring the absorption of the ferrous iron. As the pH of the bowel rises distal to the duodenum, the ferrous iron becomes autoxidized to ferric iron, decreasing the amount of iron salt capable of being absorbed. This mucosal control of iron absorption has long been held to be effective in restricting iron absorption, unless body storage depots have been depleted, but there is now sufficient evidence to indicate that the influences controlling mucosal absorption of iron can be transgressed. Under diverse conditions of experimental ferrokinetics some of the mechanisms which control the mucosal absorption mechanism have been explored.

Material and Methods

By use of the high specific activity of radioactive iron now available, an attempt to determine the precise areas of absorption of the gastrointestinal tract of the rat was undertaken by autoradiographic procedures. Iron uptake of various sections of the gastrointestinal tract and of the liver, spleen, adrenal, and kidney was determined with use of a well type scintillation counter (Tracerlab). Kodak Permeable Base Stripping Film (Experimental) was used for the anatomic demonstration of the zone phenomenon by microautoradiographs and regular clinical x-ray film for the gross topography. Groups of animals were placed on various diets in order to study the alterations in absorption.

Control Animals.—Young adult white rats from the St. Louis University colony which were placed on a normal laboratory diet received 10μc. of Fe^{59} in the ferrous ascorbate form having a specific activity of 2,130 mc. per gm. of iron and containing 0.0094 mg. of iron. The solution was administered by stomach tube. Twelve hours later the animals were killed; the gastrointestinal tract was sectioned lengthwise and washed with hydrogen peroxide in order to remove all particles of fecal material. One-half was divided into its anatomic parts, e. g., stomach, duodenum, jejunum, ileum, and large intestine, for determination of the isotopic uptake of the mucosal lining. The results of the gross autoradiographs are noted in Figure 1.

Low-Protein Diet.—A second group of animals at the weanling stage was placed on a 4% protein diet supplemented with 3% ferric ammonium citrate. Within two weeks their hemoglobin fell, and with the hemoglobin at a level of approximately 4 gm. % these animals received by stomach tube 10μc. of Fe^{59} (containing 0.0094 mg. of iron) in the ferrous ascorbate form. At stipulated periods, these animals were killed, gross organs counted, and autoradiographs prepared. These animals on a low-protein, high-iron diet showed a marked retardation in growth and considerable emaciation. Two weeks after the onset of the

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Department of Pathology, St. Louis University School of Medicine.

This paper was submitted for the Shields Warren Festschrift but was received too late to be included in the October, 1958, issue.

This investigation was supported by Grant A-1594 from the Department of Health, Education, and Welfare, U. S. Public Health Service.

Fig. 1.—Gross autoradiograph of the gastrointestinal tract from a normal rat. Stomach located in the upper right. Duodenum continuing from right to left, with jejunum, ileum, and large intestine running left to right on the bottom row. Note absorptive areas in the duodenum.

protein-poor diet the gastrointestinal absorption of Fe^{59} was studied. Within six hours the majority of the radioactive iron had been absorbed throughout all parts of the intestine (Fig. 2). The ileum and the large intestine had a count approximately six times that of the stomach, duodenum, and jejunum, the total counts for the entire gastrointestinal tract being 191,168 counts per minute (CPM). Within 12 hours the total counts in the gastrointestinal tract had dropped to 145,670 CPM, the majority of the activity being localized in the ileum. The count in the large intestine had dropped to 12,654 CPM, approximately 0.1 that of the six-hour animal, while the absorption from the duodenum had increased. Figure 3 demonstrates the localization of the isotopic iron in the distal portions of the villi of the duodenum in an unstained microautoradiograph. By the 24th hour most of the Fe^{59} had been absorbed, and the count in the stomach and the duodenum had dropped to 2,920 CPM.

Milk Diet.—The third series of animals was placed on a whole-powdered-milk diet supplemented with 5 mg. of thiamine chloride, 5 mg. of pyridoxine, and 50 mg. of calcium pantothenate per kilogram of diet. This type of diet was originally utilized by Copp and Greenberg.[8] In approximately three months, it was noted that the hemoglobin could not be reduced lower than 11 gm. (normal, 14.5 gm. %). These animals were given 10μc. of radioactive ferrous ascorbate by stomach tube and killed 12 hours later. The same procedure was followed in these animals as was followed with the animals on low-protein diets. As can be seen in Figure 4, there was an increase in absorption from the jejunum but very little from the ileum and the large intestine. Increased absorption did not take place throughout the entire tract because the iron stores of the animals were not fully depleted.

Iron-Free Diet.—The fourth group of rats was placed on a basal diet in which all iron compounds had been excluded. These animals were kept on the diet for three months before being fed the radioactive ferrous ascorbate. They were killed 12 hours after the feeding and treated in the usual manner. The hemoglobin of the animals on the iron-free diet corresponded very closely to that of the animals on the milk diet. The majority of the animals revealed a hemoglobin of 10 gm. %. The gross autoradiography of the gastrointestinal tract (Fig. 5) was similar to that of the animals on the milk diet. The Table compares the activity of the mucosal cells of the gastrointestinal tract removed from the milk-diet animals with that from

Fig. 2.—Gross autoradiograph of the gastrointestinal tract from a rat on a low-protein, high-iron diet. Stomach located at top of film, with intestine following from the right.

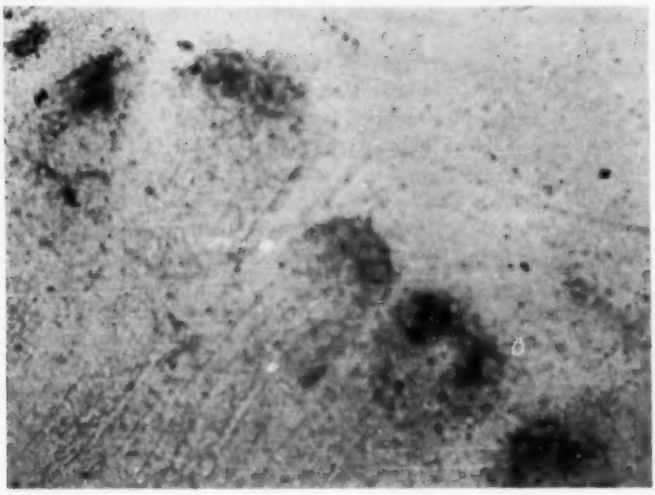

Fig. 3.—Unstained microautoradiograph of the duodenal villi from a rat on a low-protein, high-iron diet. Note reduction of film in distal portion of the villi, demonstrating presence of absorbed radioactive iron.

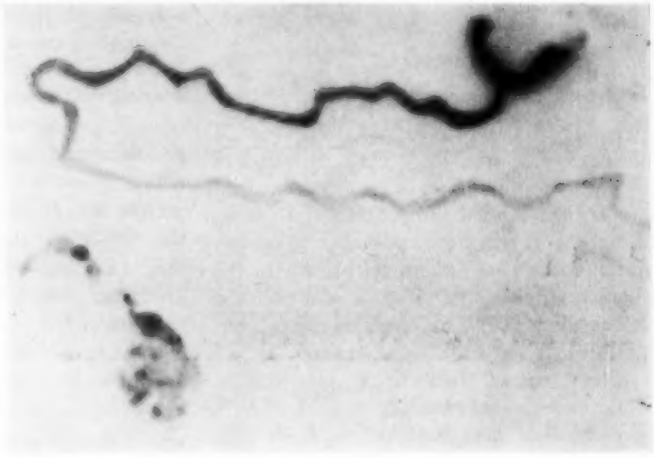

Fig. 4.—Gross autoradiograph of the gastrointestinal tract from a rat on a milk diet. Stomach located at upper right area. Note increased absorption in the jejunal area as compared to the normal.

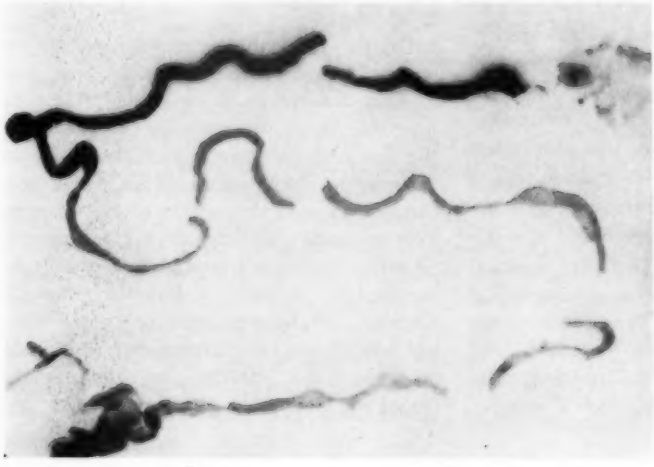

Fig. 5.—Gross autoradiograph of the gastrointestinal tract from a rat on the iron-free diet (stomach located in upper right area). Note increased absorption in the jejunal and ileal areas as compared to the normal.

Dietary Anemia

	Counts/Minute		
	Milk Diet	Fe-Free Diet	Control
Stomach	832	2,328	10,136
Duodenum	34,392	19,930	
Jejunum	12,086	11,042	6,882
Ileum	3,670	9,272	
Large intestine	15,658	3,964	8,202
Liver	2,744	8,014	5,392
Spleen	302	6,091	
Pancreas	157	1,092	
Kidney	525	4,625	1,003

the iron-free-diet animals. Slight differences are noted, and when the activity of the component parts of the gastrointestinal tract is compared with the autoradiograph, it is apparent that the increase in absorption is proportional to the hemoglobin level of these animals.

Comment

With the use of radioactive iron in conjunction with autoradiography the site of absorption of iron as a dynamic pathway of the intestinal tract was determined in the normal animal. Copp and Greenberg, using normal rats, indicated that only one-third of the radioactive iron present in the contents of the large intestine was excreted in the feces and from this evidence concluded that a considerable amount of absorption takes place in that structure. Their experimental animals on a whole-milk diet absorbed over 90% of the iron. The zones of gastrointestinal absorption of these animals was not recorded. Our observations indicate that in the normal animal absorption takes place mostly in the duodenum and the first areas of the jejunum and relatively little in the stomach. Autoradiographs illustrate this zone of iron absorption. The results of these studies indicate that in the normal animal iron absorption is restricted to the duodenal region.

With alterations in the diet and ingestion of excessive amounts of iron, an anatomical extension of the absorptive surface of the gastrointestinal tract can be produced. The animals on the milk and the iron-free diets did not present as extensive an absorptive region as the animals on the low-protein, high-iron diet. Although they had been on this diet for approximately three months, their hemoglobin did not drop below 10 mg. %. Copp and Greenberg (1946) were able to reduce the hemoglobin of their rats to approximately one-half of that of the control animals. The basic mechanism of anemia resulting from protein deficiency is not known. The difference between the hemoglobin levels in Copp's series and our own may rest upon the state of saturation of iron stores within the animals prior to the commencement of the experiment or the introduction of adventitial iron from an unsuspected source. Nevertheless, the iron demands of the anemic rats is met by gastrointestinal absorption, as shown by the radiographs and from the radioactive counts. There is a definite increase in the amount of radioactive iron in the jejunum of both groups of animals when compared to the control, but there is little absorption taking place in the stomach. Hahn [5] observed that 15% of the total dose of iron was absorbed from the isolated stomach and duodenal-jejunal pouches of the dog after the animal had been depleted of its iron stores by repeated hemorrhage. Active absorption did not occur from the remainder of the gastrointestinal tract, irrespective of the degree of anemia in which the animal was found. Exhaustion of the iron stores is apparently a mandate for intestinal absorption.

On the basis of our results, it can be stated that the stomach plays a minor role in iron absorption in reversible states of iron storage in the rat. The duodenum is the first and the main site of absorption in all animals. In the animals on low-protein diet, it is always this region of the intestine that intensifies its iron-absorption pattern. Absorption does not take place in the ileum or the large intestine unless the "mucosal block" of Hahn has been removed. This has been shown to occur not only in animals on an iron-deficient diet but also in those placed on low-protein, high-iron diets. Al-

terations from the normal in terms of depreciated hemoglobin reading, iron insufficiency, or protein depletion may remove the mucosal block. The degree of alteration determines the extent of the anatomical zone of intestinal iron absorption.[6,7]

The high iron counts in the parenchymal organs, such as the liver, kidney, and pancreas, in rats on iron-free diets in contrast with the milk-anemic rats may be a reflection of the difference between immediate marrow demands and secondary storage exhaustion.

Bethard et al.[8,9] placed a series of rats on various low-protein diets, and they believe that these protein-depleted animals may have a mechanism whereby available reserve protein may be utilized to manufacture hemoglobin when anemia is present. Sporadic bursts of erythropoiesis supported the concept of a dynamic balance between tissue and blood proteins. That protein-deficient rats cleared their blood plasma more rapidly than normal ones suggests that there is a lack of plasma protein for in vivo conjugation. This finding suggests another relationship, that iron storage may occur but in the presence of protein depletion cannot be fully utilized. The iron accumulates in depots until the protein reserve is adequate for mobilization allowing structural completion of the protein-iron complex.

Rats placed on a corn grit diet and supplemental iron absorbed large amounts of the iron in contrast to the control group.[10] These animals suffered great loss of weight, which might have resulted from the increased iron concentration of the tissues because of a decrease in tissue size and a transfer of iron from the diminishing volume of red cells. Our animals, on a 4% protein plus 3% ferric ammonium citrate diet, behaved in much the same way. The rapid rate at which the oral iron was absorbed by the intestine indicated the complete breakdown in the regulation of the absorption of the iron. The counts of all sections of our preparations of the intestines were elevated and after exposure to the x-ray film for less than a week caused maximum darkening. In those animals where great absorption is taking place, it is interesting to note that the jejunum and the ileum contain more of the radioactive iron in their mucosal cells than the duodenum. The influences controlling absorption of iron need to be evaluated in terms of dynamic cytology. How does the tagged iron penetrate the cell? Are the entrance and cytoplasmic incorporation the same in intestinal and parenchymal fields, and in the mobilization of iron does the recall of iron follow a common pathway? It is held by some investigators that a balance between the oxidizing and reducing enzymes within a cell is a major factor concerned in iron metabolism.[11]

Summary

The degree of absorption is correlated with hemoglobin value of the experimental animal. With depletion of iron stores a state of "iron want" is created in the experimental animal. This pathologic condition leads to an anatomical extension of the gastrointestinal absorptive range. Chronic anemia or protein deficiency in the experimental rat opens up the absorptive capacities of the intestinal tract anatomically far beyond the duodenal region, which is the zone of absorption under normal conditions. The site of absorption extends progressively down the intestinal tract corresponding with the diminution of the hemoglobin.

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Studies on Ferrodynamics

II. The Uptake and Distribution of Labeled Fe^{59} in Experimental Infusional Siderosis

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In humans, massive accumulations of visceral iron have been reported in two distinctive conditions: the enigmatic condition, hemochromatosis in males, and the iron-storage syndrome following repeated transfusions of whole blood.[1-3] Experimental investigation into these conditions has rested upon attempts at morphological simulation of iron storage produced in animals, such as mice, rats, and rabbits,[4-6] by intense parenteral administration of macromolecular iron compounds.

The dynamics of experimental iron storage has not been analyzed by isotopic techniques to explain the differences in cell penetration in epithelial or mesenchymal systems, the internal visceral movement of iron, or the effect of macromolecular iron upon cell enzyme systems. In this communication, the movement of iron within the metabolic cycle of the experimental animal has been studied by Fe^{59} and, in particular, the effects of blockade on the reticuloendothelial system by preparatory injections of colloidal iron. Procedures used to confirm or extend the kinetics of iron in other forms of pathological reticuloendothelial blockade were accomplished by parenteral administration of carbon black and trypan blue, producing a distinctive storage pattern in the reticuloendothelial system. A variant in blockading the marrow was attempted by total-body irradiation. In all of these experimentally induced states the intrinsic movements of tagged iron were followed.

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Department of Pathology, St. Louis University School of Medicine.

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This investigation was supported by Grant A-1594 from the Department of Health, Education, and Welfare, U. S. Public Health Service.

Materials and Methods

Young adult rabbits were the animals of choice and were killed at stipulated intervals. A series of nine control animals was given intravenous injections with 10μc. of ferrous ascorbate containing 0.0094 mg. of iron, and the iron uptake was plotted. These conrol animals were killed at ½, 1, 2, 8, 12, 24, 48, 72, and 96 hours after injection. The liver, heart, spleen, pancreas, kidneys, adrenals, femoral bone marrow, and vertebral bone marrow were removed along with blood samples for blood-protein patterns and erythrocytic uptake of Fe^{59}. The radioactivity content of these organs and samples was measured by means of a low-background well type scintillation counter (Tracerlab). Autoradiographs of the organs were prepared with use of Kodak Permeable Base Stripping Film (Experimental).

Over a period of six months, an additional group, of five rabbits, splenectomized at the beginning of the experiment in order to enrich iron storage in other organs, was given 10 ml. of a 40% solution of saccharated iron intravenously three times a week. Forty-eight hours after the last injection, 10μc of radioactive ferrous abscorbate was administered intravenously and the animals were killed and subjected to the above analytic methods.

A third group, of nine rabbits, not splenectomized, was given five injections of 10 ml. each of a 40% saccharated iron oxide solution over a period of 10 days. Fe^{59} was given intravenously 24 hours after the last dose of saccharated iron oxide solution; the animals were killed and the organs analyzed for distribution of radioactivity. Two other groups, of nine rabbits each, were given large amounts of carbon black (50 and 120 ml. of a 20% solution) and trypan blue (50 ml. of a 5% solution). A final series, of nine rabbits, received total-body irradiation of 1,000 r to destroy the reticuloendothelial system. These animals were killed at definite intervals after they had received

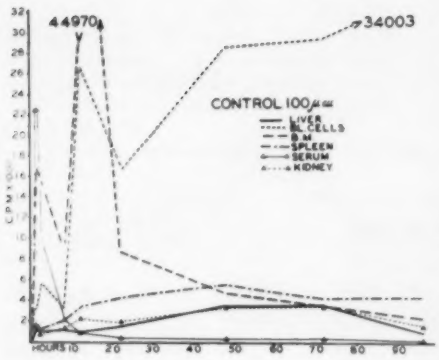

Fig. 1.—Normal uptake of tracer dose of radioactive iron. Organs have been placed on a per-gram-weight basis.

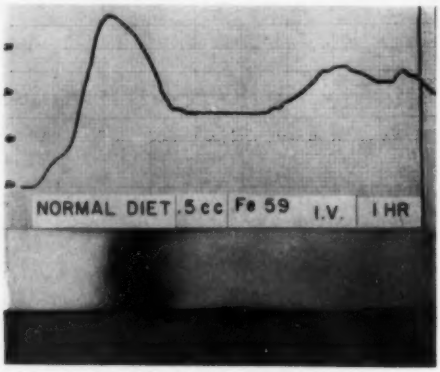

Fig. 2.—Electrophoretic serum pattern of normal rabbit blood. Autoradiograph; lower strip demonstrates localization of radioactive iron in the β-fraction of the serum globulin.

the tracer dose of elemental radioactive iron and treated similarly to the iron overload animals. A total of 50 rabbits was used in this experimental investigation.

Results

In the control animals (Fig. 1) it was noted that the small amount of radioactive ferrous ascorbate (0.0094 mg. of iron) given intravenously followed the well-known behavior pattern of normally absorbed iron. The serum readily bound the iron; Figure 2 demonstrates the electrophoretic pattern of the serum protein from the radioactive samples. The electrophoretic strip was placed on clinical x-ray film and exposed for one week. The lower strip of Figure 2 is the developed x-ray film. The dark areas show the iron contained in the β-fraction of the serum globulin. Within two hours after the tracer dose the serum radioactive-iron content fell, dropping to insignificant amounts within 20 hours, while the bone marrow activity rose rapidly within 8 hours, reaching its peak within 12 hours. At that time the radioactive counts of the bone marrow registered a 100% increase over the serum at its highest concentration. Within 12 hours after injection, the cellular components of the blood contained a significant concentration of the isotopic iron. After 96 hours the majority of the radioactivity had disappeared from the organs, while the red cell generation continued in its incorporation of all the radioactive iron. At this time 72% of the total activity was present in the erythrocytes.

In the animals that had received the massive doses of saccharated iron oxide over a period of six months it was noted that within eight hours after the administration of the tracer dose there was a definite iron uptake in all of the organs, with

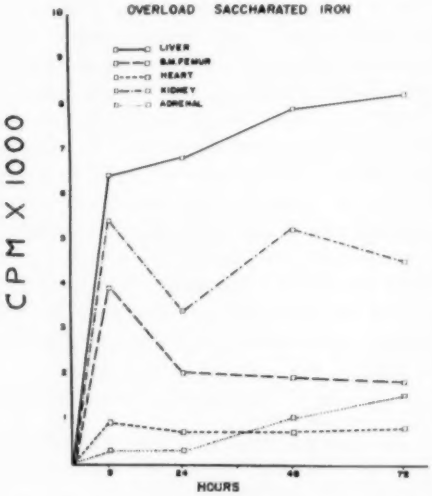

Fig. 3.—Uptake of tracer dose of radioactive iron in the overloaded animals. Organs have been placed on a per-gram-weight basis.

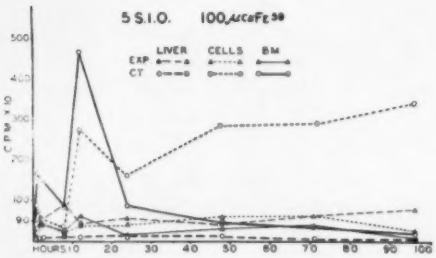

Fig. 4.—Comparison of the liver, erythrocytes, and bone marrow in rabbits receiving five injections of saccharated iron oxide.

the greatest being in the liver, the kidney, and the heart, in that order (Fig. 3). By the end of 24 hours, all of the organs had lost approximately 50% in radioactivity, with the exception of the liver which continued to concentrate the iron as indicated by the increments in count. The inference is that the radioactive iron in organs other than the liver is merely transient. The injected nonradioactive saccharated iron oxide more than supplied the iron wants of the body. Consequently, once these immediate iron demands were met, the remainder of the injected iron was deposited in the liver.

The animals which received five injections (50 ml.) of saccharated iron oxide (625 mg. of elemental iron) were administered 10μc. of radioactive ferrous ascorbate intravenously, and the organ analysis showed approximately the same pattern of activity as in the animals which had received over 70 injections, 1.5 gm. of elemental iron (Figs. 4 and 5).

In an effort to determine the precise amount of iron necessary to satisfy and hold the basic demands of the marrow with the shunting of the excess to the liver, a series of rabbits was given graded amounts of 40% saccharated iron oxide containing 12.5 mg. per milliliter of elemental iron labeled with a tracer dose of radioactive iron. It was determined that in the normal adult rabbit 4.7 ml. of a 40% solution of saccharated iron oxide (58.8 mg. of elemental iron) satisfied the marrow demands for iron. All iron subsequently injected into the animal was deposited in the liver. Although the activity in the adrenals was small, when they were placed on an activity-per-gram-weight basis they showed a graduated increase, closely approximating the bone marrow at the end of 72 hours.

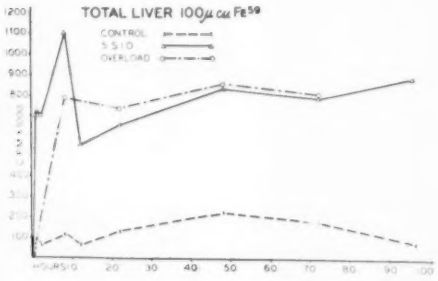

Fig. 5.—Comparison of the liver uptake of saccharated iron oxide in the overloaded, the five injected, and the control rabbits.

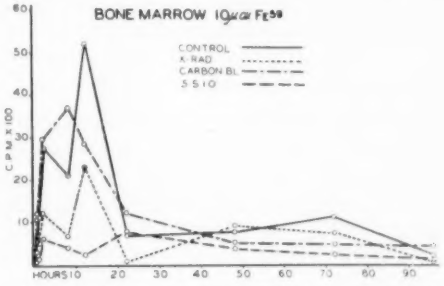

Fig. 6.—Bone marrow uptake in the control, x-irradiated, and carbon black-loaded rabbits and in rabbits receiving five injections of saccharated iron oxide.

Figure 6 is a graph of the bone marrow uptake of the rabbits over a period of 96 hours while receiving 50 ml. of saccharated iron oxide (625 mg. of iron), carbon black, and ionizing radiation to the marrow. As compared to the control, very little of the tracer dose passed through the bone marrow after its wants were supplied by the saccharated iron oxide. Carbon black (Fig. 7) did not interfere with the marrow uptake of iron, and shunting of iron into the liver depot was not encountered. X-radiation (Fig. 8) suppressed bone marrow uptake of iron to a degree but not to the extent

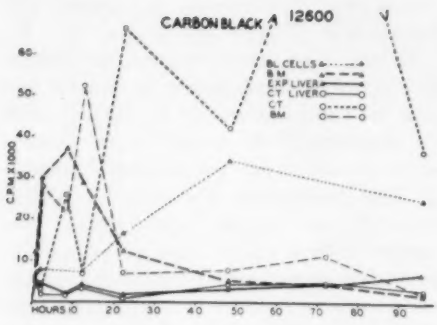

Fig. 7.—Uptake of tracer dose of iron in the liver, erythrocytes, and bone marrow in animals loaded with carbon black, compared with controls.

observed in the animals receiving saccharated iron oxide.

The blood cells follow the same pattern, although the activity curve of the blood of the x-irradiated animals is very similar to that of the overloaded animals.

Comment

Massive amounts of injected iron rapidly supply the demands of the erythropoietic system and open up the liver as a storage depot. After these demands were met, all of the radioactive tracer dose was evenly distributed throughout the peripheral portions of the liver lobules.

These findings agree with those of Dubach et al.[7] in that there is so much untagged available iron from daily breakdown of red cells and the storage depots that the body rarely utilizes the labeled iron. The minute quantity of radioactive iron is widely spread throughout the liver.

The minute quantities found in the blood cells of the overloaded animals remain constant, indicating a small and gradual utilization of iron over extended periods of time. This is in contrast to the control animals, in which the tagged iron was immediately incorporated in the erythrocytic cycle after injection in preference to the normal iron stores of the animal.

In this paper the evidence is supplied that the injected iron not only supplied the erythropoietic system but saturated the body to a point where it would accept iron only to a limited degree, the one exception being the liver. This organ continues to concentrate the iron with seemingly no alterations in its function.

Histological appearance showed the cardinal features of ferric thesaurosis.[8] The kidneys of the overloaded animals showed a capillary engorgement of the glomeruli, trapped iron particles in the capillaries, and dispersed granules of iron prominently displayed in the proximal convoluted tubules. The distal convoluted tubules were clear. Foci of swollen macrophages with numerous granules of iron were found in the bone marrow, giving a moderate Prussian blue reaction. The sinusoidal cells of the lymph nodes contained an increase in concentration, whereas in the adrenals there was a moderate amount of iron localized in the cells of the perimedullar zone. Sections of the heart demonstrated a scattering of intramuscular hemosiderin granules. In the liver there was a preponderance of iron localized in the Kuppfer cells of the reticuloendothelial system, with a peripheral distribution in the parenchymal cells of the lobules (Figs. 9 and 10).

The data presented here show that in the "overloaded" animal the intravenously injected radioactive iron goes directly to the

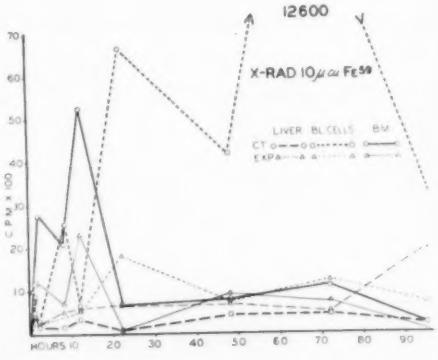

Fig. 8.—Uptake of tracer dose of iron in the liver, erythrocytes, and bone marrow in animals that had received 1,000 r, compared with controls.

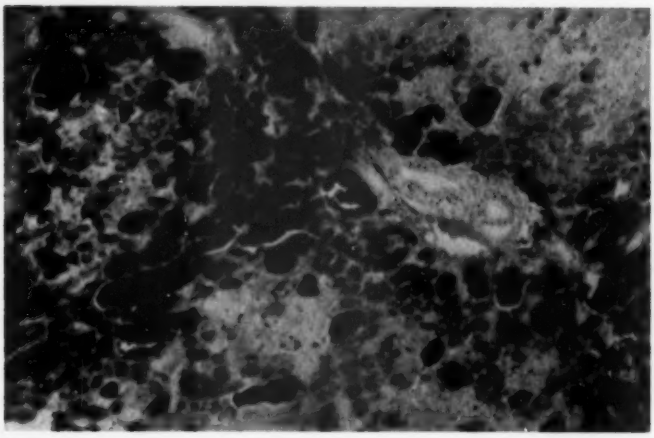

Fig. 9.—Section of liver from a rabbit that had received massive doses of saccharated iron oxide. Prussian-blue reaction; reduced 10% from mag. × 220.

liver. This organ demonstrated radioactivity approximately 100 times that of the bone marrow. On a per-gram basis it is noted that the concentration of radioactivity within the liver continued to rise for 72 hours after injection. In the liver not only are the reticular elements involved in storage but the almost limitless thesauric properties of the parenchymal cells have to be considered. Kidney and bone marrow followed next in magnitude. Heart and skeletal muscle showed limited degrees of radioactivity under these experimental conditions. It is interesting to note that in the adrenals, although the radioisotopic iron accumulation was not great, there was a progressive increase up to 72 hours, at which time the concentration, calculated on a gram basis, was approximately that of the femoral bone marrow.

In the consideration of radioisotopic uptake within the reticuloendothelial system, it should be noted that this system is extremely labile and in a constant state of activity.[9] Morphologic charging of the reticuloendothelial system with such substances as carbon black and trypan blue does not greatly alter the ability of the body

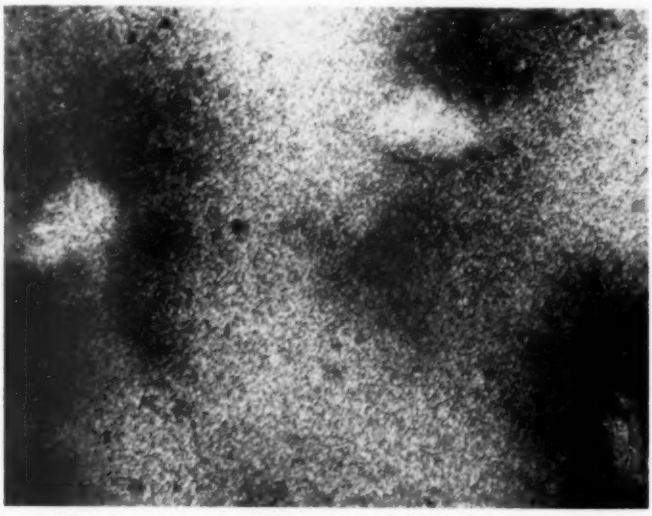

Fig. 10. — Microautoradiograph of the liver of the rabbit demonstrates localization of radioactive iron in the peripheral portions of the lobules.

to handle iron. These amounts of carbon black and trypan blue given over a period of several weeks were not detrimental to the physiological handling of body iron. The reticuloendothelial cells of the body were definitely stimulated, but their proliferative capabilities had not interfered with their function as far as iron metabolism was concerned.

With x-radiation, although the evidence of histological destruction was widespread, the functional ability of the marrow was partially retained. These findings, on the basis of handling and "clearance" of the Fe^{59}, would suggest that the conventional histological appearance of reticuloendothelial "blockade" may be a false facade. The cells of such a system, appearing to be morphologically loaded, might even be capable of exalted function. Only when the agent is directly concerned with enzymic cellular function is a complete functional block established.

Conclusions

A "functional blockade" of the bone marrow is readily produced with intravenously administered saccharated iron. With the establishment of such a state, the excess of the administered iron is deposited and rapidly accumulates within the liver; the liver sits at "first table" as far as consumption of excess iron is concerned. This labeled iron is deposited initially at the peripheral zones of the liver lobule and with increasing concentrations spreads diffusely throughout the lobule. Fresh and old iron may occupy the same cellular site. These experimental findings offer an explanation for the massive amounts of iron recovered from the liver in hemochromatosis and the relatively limited concentration of iron noted in the marrow in this condition. Dyes phagocytosed by the reticuloendothelial system or ionizing radiations applied to the marrow do not produce a blockade comparable to that observed with focal concentrations of polymeric iron within the marrow. Histological appearance of the marrow, except for the demonstration of stainable iron, may be spurious as far as presenting a valid indication of functional activity. To produce successively a "functional blockade" of marrow as far as iron is concerned requires a critical dose of an acceptable iron compound; substitutes of unrelated agents, even though phagocytosed by the reticuloendothelial system, were of little value. Necrotizing agents of a selective type may be of greater value in the production of marrow dysfunction in terms of ferrodynamics.

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Gastric Mucormycosis in a Newborn Infant

A Case Report

ROBERT R. GATLING, M.D., Roanoke, Va.

Mucormycosis of the digestive tract is an infrequently reported entity. Its presence in the absence of diabetes or antecedent debilitating disease appears to be even less frequent. Baker et al.[1] were able to collect only three cases from the literature prior to February, 1957, and reported two of their own. Since that report, Watson [3] reported a case occurring in a 26-month-old infant who also had kwashiorkor. Visceral disease due to mucor is of particular significance in the light of the present unsatisfactory status of therapy and an incomplete understanding of its pathogenesis. Baker [2] indicated that antibiotic therapy might possibly play a role and wrote, "The improvement in the control of bacterial diseases by the use of antibiotics may explain why mucormycosis is more frequent."

The purpose of this paper is to report a case of gastric mucormycosis occurring in an otherwise apparently healthy newborn infant who died of the infection on the 16th day.

Report of Case

Summary of the History

This white girl was the product of an uneventful full-term pregnancy of a 32-year-old primipara. The membranes were ruptured surgically, and labor was instituted by means of an intravenous drip of oxytocin (Pitocin). The first stage of labor lasted eight hours, and delivery was facilitated by means of cyclopropane anesthesia and low forceps. The birth weight was 2,965 gm. A physical examination was carried out shortly after birth, and no abnormalities were found. The cry was described as "lusty."

Submitted for publication July 31, 1958.

From the Department of Pathology, Lewis-Gale Hospital.

Laboratory Data

Four days after birth the volume of packed red cells was 74%; the hemoglobin was 23 gm. %, and the white cells numbered 15,600. Of the total white cells, 58% were neutrophils; 35%, lymphocytes; 5%, eosinophils, and 2%, basophils. The hemoglobin gradually decreased to 18 gm. % by the 15th day. Stool culture made on the 12th day produced no growth. On the 6th day, the spinal fluid sugar was 48 mg. % and there were many red cells (bloody tap); on the 10th day, the spinal fluid protein was 38 mg. %, and, on the 15th day, the spinal fluid contained 25 mg. % protein, 63 mg. % sugar, and 15 white cells per cu. mm.

Course in the Hospital

During the first four days after delivery the baby took its formula and had normal bowel movements and normal urine excretion. On the fifth day it was noticed to be fussy, and the rectal temperature rose to 101.8 F. Chloramphenicol (Chloromycetin), 200 mg. intramuscularly every 12 hours, was begun. On the sixth day, the left tympanic membrane appeared dull and a myringotomy was performed, which resulted in a small amount of mucoid drainage. Hemolytic Staphylococcus albus was cultured from the left ear canal. (The coagulase test was not carried out.) On the sixth day, also, the abdomen was noted to be distended for the first time and gastric suction was instituted. X-rays of the skull and chest at that time were normal. A flat film of the abdomen demonstrated adynamic ileus. Roentgenographic examination of the colon revealed no abnormality. On the seventh day, the infant began to vomit and had a spell of cyanosis. Slight drainage from the left ear canal continued. Combined penicillin and dihydrostreptomycin (Combiotic), 2 cc. intramuscularly daily, and nystatin (Mycostatin) suspension, 1 cc. t. i. d., were added to the therapeutic regimen. Two cubic centimeters of γ-globulin was given on the 6th, 10th, and 15th days respectively. Periodic vomiting and spells of cyanosis continued. Diarrhea began on the ninth day. These symptoms persisted; the baby's condition gradually worsened, and she died on the 16th day.

Autopsy Report

Autopsy was begun two and one-half hours post mortem. The body was that of a well-developed and fairly well-nourished white girl appearing to be about the stated age of 16 days. The head measured 31 cm. in circumference; the chest, 23.5 cm., and the abdomen, 34.5 cm. The crown-heel length was 45 cm. No rigor mortis was present. There were no skin rashes, ulcers, or petechiae. Several needle-puncture wounds were present in the skin of the buttocks. There was no subcutaneous lymphadenopathy. The umbilical cord stump was dry and shriveled. The head was symmetrical. The pupils were equal and regular and measured 0.4 cm. each. There was no discharge from the ears, nose, or mouth. The lips and oral mucosa appeared cyanotic. The abdomen was considerably distended. The external genitalia were normal. The anal canal and rectum were patent, and no blood or tarry fecal material was present. The peritoneal cavity contained approximately 75 ml. of clear straw-colored fluid. The serosa of the stomach was adherent to the colon, liver, spleen, left leaf of the diaphragm, and anterior abdominal wall by means of fibrinous adhesions. A walled-off collection of cloudy fluid was present beneath the dome of the diaphragm on the left. There were numerous enlarged mesenteric lymph nodes measuring up to 1 cm. in diameter. No measurable free fluid was present within the pleural cavities. The base of the left lung was adherent to the diaphragm by fibrinous adhesions. The size of the thymus gland appeared greatly diminished. No excess of free fluid was present within the pericardial cavity, and the surfaces were smooth and glistening.

The heart weighed 20 gm. There were no abnormalities. The aorta was normal.

The right lung weighed 24 gm., the left, 20 gm. Both lungs were deep red and appeared poorly expanded. There were small pink-tan areas of expanded lung along the anterior border of each. The pleural surface of the left base was covered with fibrin, as was the adjacent diaphragmatic pleura. The cut surfaces of the lungs were deep red generally, but the left base was hemorrhagic.

The entire bowel appeared moderately distended with gas and contained a small quantity of greenish liquid. The wall of the stomach, from the cardia to within 0.5 cm. of the pylorus, was greatly thickened and indurated (Fig. 1). The mucosa of the stomach was ragged and tan-brown and appeared somewhat opaque, but this alteration ceased abruptly at 0.5 cm. proximal to the pylorus. The serosal surface was covered by fibrin. The wall of the transverse colon adjacent to the stomach was thickened and covered with fibrin.

The liver weighed 210 gm. There was a 2×5 cm. oval, dull, green-brown area of alteration of the surface which lay in contact with the stomach. The cut surface of the liver underlying the latter area was gray for a depth of 0.4 cm., but the liver elsewhere was red-brown and appeared normal.

The spleen weighed 12 gm. and was largely incorporated within the exudate beneath the left leaf of the diaphragm. It was deep red and firm.

The pancreas appeared normal in size, but the lobules did not move readily. There were several

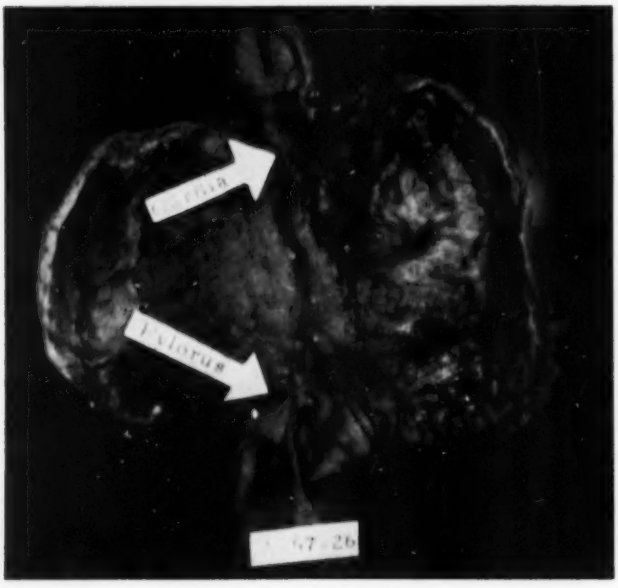

Fig. 1.—Stomach opened along greater curvature. The sharp demarcation of the exudate at the pyloric end is evident.

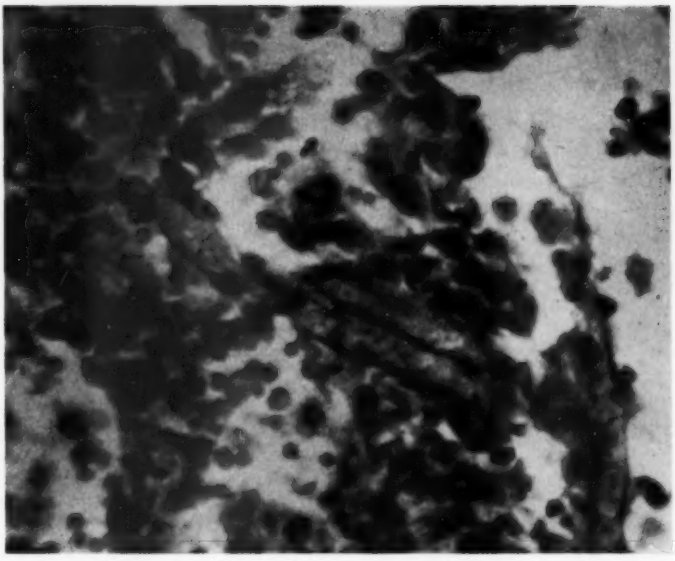

Fig. 2.—Lung. Hyphae lie within the alveolar spaces and extend through the septa. Hematoxylin and eosin; reduced 1/3 from mag. × 650.

small, focal, chalky areas of fat necrosis over the anterior surface. The peripancreatic connective tissue appeared edematous.

The adrenals appeared normal.

The kidneys weighed 15 gm. each and were red-purple. Scattered petechial hemorrhages were visible through the capsule. The capsules stripped easily, revealing prominent fetal lobulations but otherwise perfectly smooth surfaces. The cortex of each measured 0.3 cm. in thickness. The cortical striations were prominent. The glomeruli were visible but pale. The periureteral connective tissue on the left was very edematous. The internal genitalia appeared normal.

The brain was not examined.

Bacteriology

Cultures of the gastric contents and the subdiaphragmatic abscess both produced a heavy growth of Friedländer bacilli. The cultures were transferred to Sabouraud's media after four days, but these, too, were overgrown by the Friedländer bacillus.

Microscopic Examination

No abnormalities of the heart or aorta were present. Deposits of fibrin were present upon the pleural surface of the left lung base, and the adjacent alveoli and interstitial tissue contained frequent large nonseptate hyphae (Fig. 2). The hyphae were invading the vessel walls, and an occasional vessel was thrombosed. Fresh hemorrhage had occurred into this area. The fungi appeared to be extending into the lungs from the basal pleural surface. The alveolar walls of numerous areas of both lungs were thickened and contained many mononuclear cells (Fig. 3). Numerous bodies, which stained deep rose with the Gridley stain, were present in alveolar septa in these areas. The alveoli of both lungs and connective tissue about the bronchi and trachea contained numerous mast cells; these stained faintly, if at all, with the Gridley stain.

The gastric mucosa had undergone extensive liquefaction necrosis. Large numbers of nonseptate hyphae were present within the mucosa and extended through the entire stomach wall. There was extensive vascular invasion (Fig. 4). The infection had extended within the wall beneath the intact mucosa at the pyloric end. Large numbers of mast cells and many mononuclear cells, granulocytes, and spores were present throughout the wall, and they will be discussed later. Both the Gram stain and the Goodpasture stain revealed

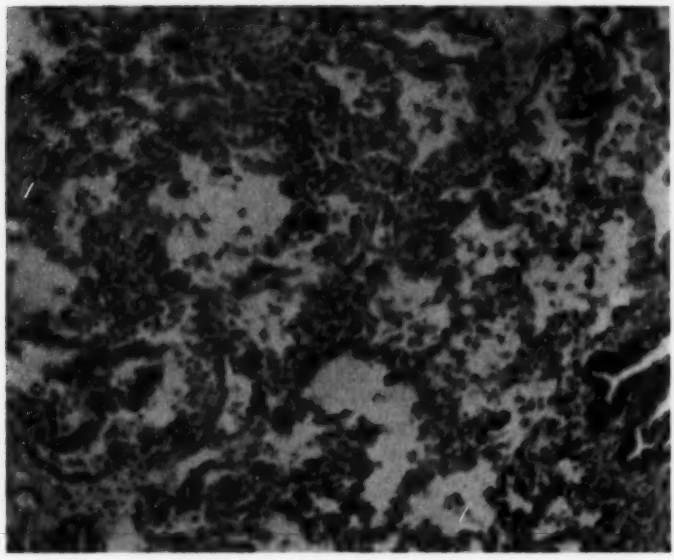

Fig. 3.—Lung demonstrating interstitial exudate and mast cells within alveoli. Rounded bodies, similar to those demonstrated in Figure 7, were present within the septa in areas such as this. Hematoxylin and eosin; reduced 1/3 from mag. × 145.

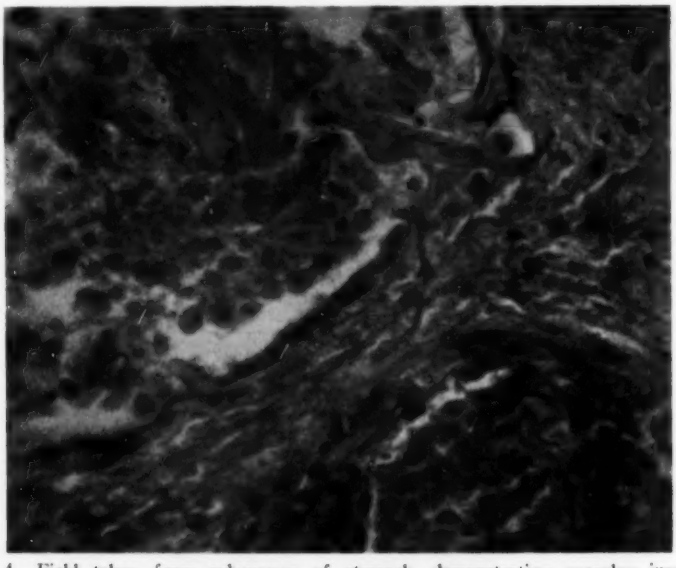

Fig. 4.—Field taken from submucosa of stomach, demonstrating vascular invasion by hyphae. Hematoxylin and eosin; reduced ⅓ from mag. × 650.

many Gram-negative bacilli and occasional Gram-positive cocci within the superficial portion of the necrotic mucosa.

The altered area of the liver in contact with the stomach was invaded by the fungus by direct extension in a manner similar to the invasion of the lung (Fig. 5). The remainder of the liver appeared unaltered.

The hilus of the spleen, the anterior surface of the pancreas, and the transverse

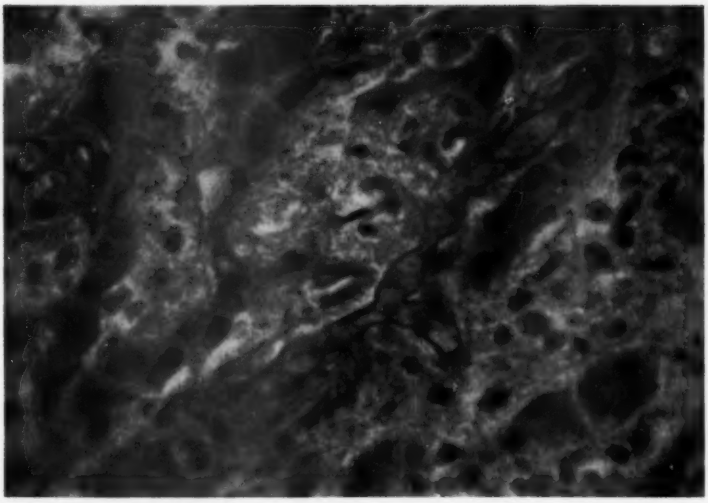

Fig. 5.—Liver. Nonseptate hypha is present in center of field. Hematoxylin and eosin; reduced 1/3 from mag. × 650.

colon appeared to be invaded by continuity from the stomach (Fig. 6). The mucosa of the colon overlying the involved portion of the wall was not ulcerated. Deposits of fibrin were present upon the serosal covering. The musculature of the left leaf of the diaphragm was diffusely infiltrated by the fungus. No hyphae were identified within the enlarged mesenteric lymph nodes. The lymph sinuses of the nodes, the periadrenal fat, the submucosa of the renal pelves, and the connective tissue about the

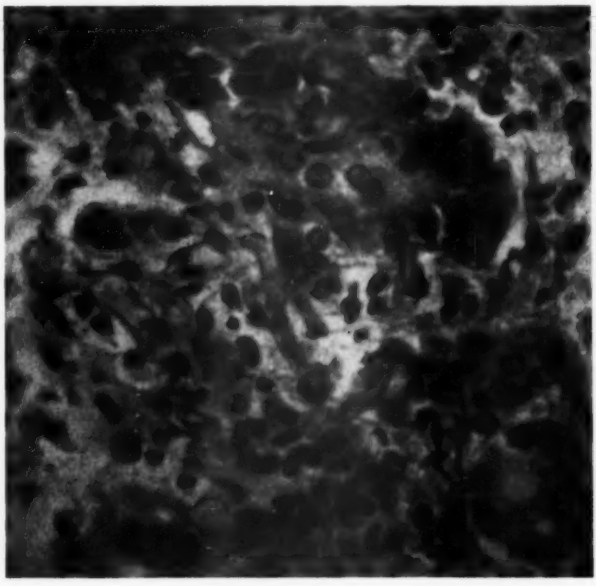

Fig. 6.—Pancreas. Numerous hyphae are scattered among the acini. Lymphocytes and monocytes are numerous. Hematoxylin and eosin; reduced 1/3 from mag. × 650.

bladder contained numerous cells resembling mast cells. The internal genitalia were not altered.

The nuclei of the thymocytes appeared small and pyknotic. Hassall's corpuscles appeared extensively degenerated.

The bone marrow was 100% cellular. The megakaryocytes were numerous.

Anatomical Diagnoses

1. Acute necrotizing phlegmonous gastritis due to mucor, with extension to the liver, spleen, pancreas, colon, diaphragm, and left lung.

2. Interstitial pneumonia, probably due to mucor.

Comment

This case is unusual in that it occurred in an otherwise normal newborn infant. This seems to be the youngest patient in whom the disease has been reported. Jackson and Karnouchow [5] reported a case of cerebral mucormycosis occurring in a 20-day-old infant.

The relationship, if any, of the otitis media has not been ascertained, nor has its relationship to the Friedländer bacilli cultured from the stomach and subdiaphragmatic abscess; indeed, there is no proof as to which was present first. Prior to the ear infection the baby appeared to be entirely normal, and the abdominal symptoms appeared only two days after the discovery of a dull ear drum. Since only 24 hours lapsed between the commencement of antibiotic therapy and the onset of abdominal symptoms, it seems unlikely that this treatment played a role. There is no information available that might indicate a source of the infection. Even though nystatin (Mycostatin) was begun 24 hours after the abdominal symptoms, there is no evidence that the course was altered thereby. The absence of glycosuria on the fourth day is evidence that diabetes was probably not a factor. Even though the brain was not examined at autopsy, the normal spinal fluid, except for slight increase in cells, on the day of death is regarded as evidence against significant central nervous system involvement.

The stomach has been regarded as probably the primary site of the infection because of the greater extent of involvement of this organ and because it seemed to be the center of the centrifugally spreading disease.

The characteristic invasion of the walls of the blood vessels occurred in this case as it has been emphasized in previous reports.[1,2] The pattern of spread appeared to be by direct extension through the connective tissues and through vessel walls, but transport of the fungus by the flow of blood and lymph did not seem to be a means of spread, with the possible exception of the areas of interstitial pneumonia. No evidence has been found of extension of the infection over the mucosal surface in this case; on the contrary, it appeared to spread beneath the intact mucosa of the stomach and esophagus without initial mucosal alteration. The mucosa seemed to be invaded secondarily. Preceding the spread of the fungus, the tissues appeared to be studded with mast cells.

The stomach wall contained large numbers of mononuclear cells and granulocytes within which or superimposed upon which were many PAS-positive bodies which stained deep rose with Gridley's fungus stain. These were of variable size, varying from the size of a mast-cell granule to 2μ or 3μ in diameter. An occasional body was observed to be budding (Fig. 7). Although many of the mast-cell granules were swollen to considerable size and appeared to be extruded into the surrounding tissue, the bodies alluded to above did not seem to be mast-cell granules, for the latter stained very poorly, if at all, with the Gridley stain. Occasional small hyphae appeared to be growing out of, or at least adjacent to, a cell containing these bodies. These bodies appear to be spores, and the presence of an occasional budding form seems to support this concept (Fig. 7). There is a

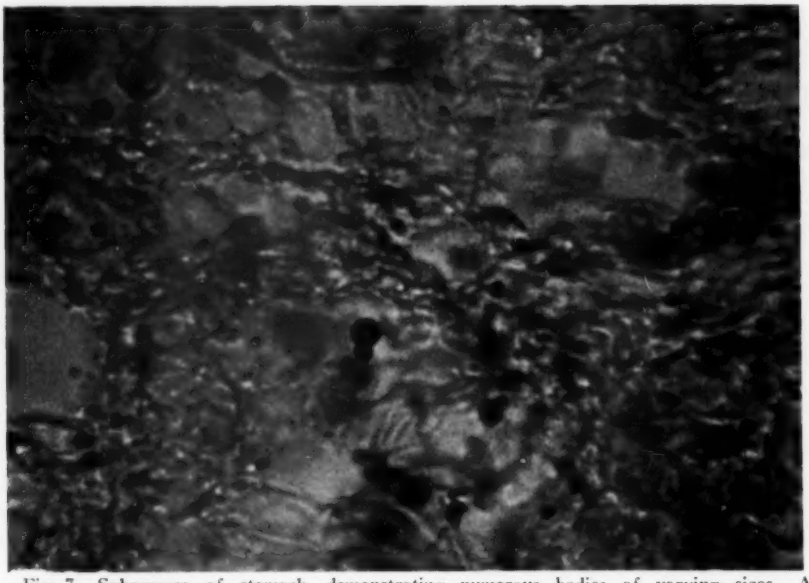

Fig. 7.—Submucosa of stomach, demonstrating numerous bodies of varying sizes. A budding form is present in the center of the field. Gridley stain; reduced ⅓ from mag. × 1,440.

possibility that the phagocytic cells may have played an important role in the spread of the fungus. In support of this concept is the pattern of spread directly through the tissues and not along the mucosal surfaces. There also seems to be a possibility that the large numbers of mast cells, with the swollen granules, may have played a role in the spread of the spores and the migration of the phagocytes. If the number and size of the mast-cell granules may be considered an index as to the quantity of heparin liberated, the latter may have occurred in sufficient quantity to interfere significantly with fibrin formation and thereby remove an important barrier to the spread of infection through the tissues.

The presence of frequent bodies resembling spores within the alveolar septa in the areas of the interstitial exudate may indicate that this agent is capable of producing interstitial pneumonia. No previous reports are found which describe interstitial pneumonia as a lesion produced by mucor.

Summary

A case of fatal mucormycosis occurring in an otherwise normal 16-day-old infant is reported. The infection appeared to have begun in the stomach and to have spread extensively to adjacent organs by direct extension. Evidence is given to suggest a role played by the macrophages and the mast cells in the spread of the disease. The possibility is entertained that mucor may be another agent which is capable, under proper conditions, of causing interstitial pneumonia.

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Irradiation Injury to the Aorta an the Lung

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Although injury to the lung in the course of irradiation is a well-described entity, from an extensive review of literature this appears to be the first report of a clearly defined aortic injury which is directly attributable to irradiation.

Report of a Case

The incident occurred in a 29-year-old unmarried white man, a school teacher, whose previous illnesses consisted of nothing more than mumps, orchitis, and herpes zoster.

When this man was seen at The Duke Hospital for the first time, on Aug. 14, 1957, his complaints were fever, nausea, vomiting, and weakness which had existed for three weeks. However, the patient's illness actually dated back two and one-half years, when he developed weakness, easy fatigability, and bilateral hilar lymph node enlargement. At that time a lymph node was removed from the right supraclavicular area, and the diagnosis "malignant lymphoma possibly of giant follicular type" was made. On the basis of this he had been given a course of deep x-ray treatment. Total irradiation of the hilar lesion was estimated to be 1,860 r. He had received more irradiation (quantity unknown) one year prior to admission to The Duke Hospital. Four months before this admission his initial complaints recurred. When his physician examined him, he found some congestion of the face and neck, suggesting a superior vena caval syndrome. Sternal bone marrow was hypoplastic owing presumably to the previous deep x-ray therapy. Following this examination, the patient was given 24 mg. of mechlorethamine hydrochloride (nitrogen mustard) in four divided doses and another course of deep x-ray therapy (about 1,000 r). His condition gradually deteriorated as indicated by loss of appetite, inability to take solid food, choking sensation on swallowing, vomiting after taking food, and excessive nonproductive cough. He lost 10 lb. in three weeks and had difficulty in getting up from bed without help. Before admission to The Duke Hospital he had been treated with a variety of drugs, tetracycline (Achromycin), penicillin, chloramphenicol (Chloromycetin), propantheline (Pro-Banthine), novobiocin (Albamycin), and saturated solution of potassium iodide (SSKI), in addition to the altogether unknown quantity of irradiation.

When admitted to our hospital the patient had cyanosis of the lips and nail beds. His pulse was 122, and his respirations, 28. His temperature on admission was 104 F; blood pressure in the left arm was 90/45. There was dullness at the base of both lungs, associated with loud and harsh breath sounds. Chest film showed greatly increased density in both lung fields compatible with tumor infiltration, leaving only a small amount of normal lung parenchyma at the bases. Sinus tachycardia and a soft systolic heart murmur completed the significant physical findings.

Laboratory studies provided the following data: Hb. 16.3 gm. %; WBC 21,500 per centimeter, with neutrophilic polymorphonuclear leukocytes 82%, small lymphocytes 10%, monocytes 1%, stab cells 7%, hematocrit 49.5%; skin tests with tuberculin, blastomycin, coccidioidin, and histoplasmin, negative; VDRL test nonreactive; stool and urine normal; blood sugar 110 mg. %; N. P. N. 52 mg. %, sodium 136 mEq., potassium 4.4 mEq., CO_2 combining power 28.5 mEq., phosphorus 3.3 mg. %; blood uric acid 5.2 mg. %; sputum and blood culture negative.

Because of increasing dyspnea and cyanosis associated with wheezing and rhonchi in both lungs, the patient had to be kept continuously under oxygen. He died 11 days after admission, which was approximately two and one-half years after the diagnosis of the disease for which he was treated by irradiation.

Autopsy.—Autopsy performed five hours after death led to the following anatomical diagnosis: X-irradiation injury to the lung resulting in pulmonary edema of high-protein type, focal necrosis and disruption of alveolar walls with microscopic cyst formation, and diffuse progressing interstitial pulmonary fibrosis; x-irradiation injury to thoracic aorta resulting in necrosis of all layers of the aortic wall followed by aortic thrombosis, fibrous hyperplasia of the intima and adventitia, and round-cell

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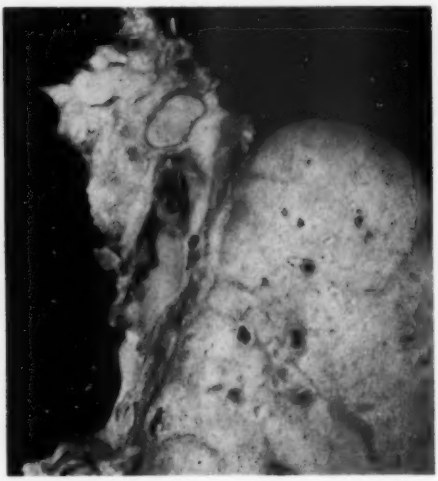

Fig. 1.—Gross appearance of the irradiated lung and the adjacent mediastinal mass. Gray fairly uniform consolidation with small cyst-like spaces characterize the lesion. Dense fibrous tissue and an area of necrotic lymphoid tissue are the only remains of the original mediastinal mass. See Figures 2 and 3 for details of the pulmonary lesion.

infiltration about the adventitial vasa vasorum; eradication of all traces of a mediastinal lymphomatous mass (specific diagnosis doubtful) by thoracic irradiation, accompanied by destruction of all neighboring lymphoid tissue; hypoplasia of the sternal bone marrow due to thoracic x-irradiation; infarction in the kidneys and spleen due to arterial emboli originating at the site of aortic irradiation injury; adenoma of the liver.

Lungs, Bronchi, and Trachea: The trachea and bronchi showed slight congestion and no obstruction. There was no fluid in the pleural cavities. One small pleural adhesion was present at the apex of the right lung. The left lung weighed 1,175 gm., and the right lung, 1,300 gm. There were small hemorrhages on the pleural surfaces of both lungs. The pleura was not thickened. Cut surface showed a remarkable paucity of air in both lungs (Fig. 1). However, both lungs were well expanded but felt heavy and fleshy. There was some edema fluid. No discrete nodules were present. The cut surface was grayish red, with scattered small and large patches of hemorrhage. The alterations of pulmonary structures were uniform throughout all lobes of both lungs. Some mucus was present in the main bronchi. The pulmonary vessels showed no significant alterations. Attached to the posterior border of the lower lobe of the right lung was a small nodule measuring $0.5 \times 0.5 \times 2$ cm. which on section was red and spongy and firmly attached to the pleura.

As observed under the microscope, the pulmonary alveoli and bronchi were filled with a most peculiar pink granular material which usually was vacuolated but sometimes was solid (Figs. 2 and 3). The solid form in some places lined the alveoli as a hyaline membrane. This material stained light brown with phosphotungstic acid hemotoxylin. It gave a positive reaction with periodic acid-Schiff stain. Congo red stain for amyloid was negative. Oil red staining revealed some fat in the pink

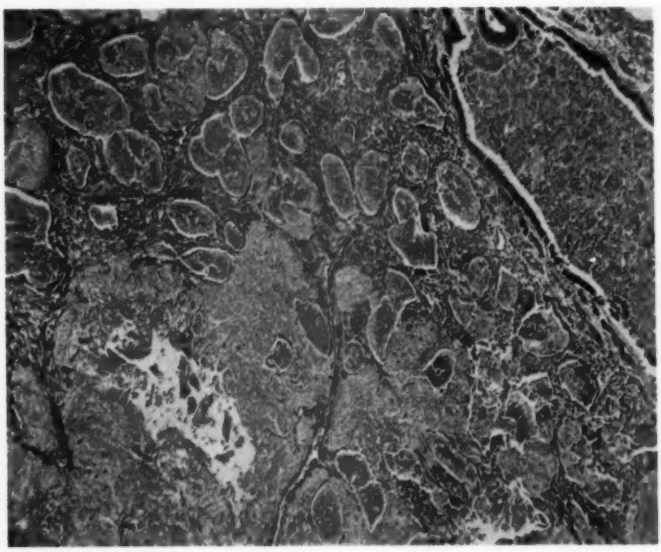

Fig. 2.—Photograph of a section from a block of irradiated lung such as that represented in Figure 1. The peculiar proteinaceous exudate in the alveoli, necrosis of alveolar walls with the formation of cysts, interstitial fibrosis, and round-cell infiltration are the characteristic features of the lesion.

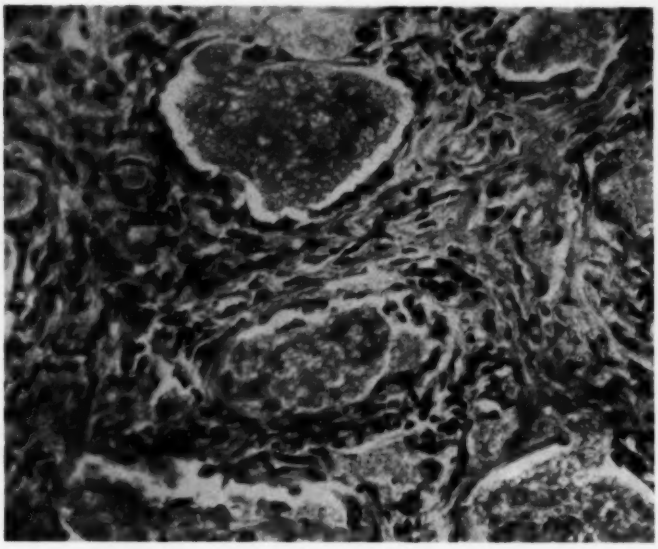

Fig. 3.—An area of advanced alteration of the lung comparable to that in the upper half of Figure 2, magnified to show the progressing interstitial fibrosis and the peculiar hyaline vacuolated granular structure of the alveolar exudate.

debris as well as in macrophages in the alveolar walls. The MacCallum-Goodpasture stain for bacteria and the Gridley stain for fungi were negative. No iron was demonstrated by the Prussian blue technique. No air-containing alveoli were present in some of the sections. Epithelization of alveoli was a prominent feature. Alveolar walls were greately thickened and with Masson's trichrome stain showed a conspicuous increase in fibrous tissue (Fig. 3). Infiltration by lymphocytes, plasma cells, and foamy macrophages was prominent. Some of the macrophages contained periodic acid-Schiff-positive material. In some areas the alveolar walls had disintegrated, resulting in cavities measuring up to 5 mm. (Fig. 2). Verhoeff-Van Gieson stain showed fragmentation of elastic tissue in some of the alveolar walls and complete disappearance of elastic fibers where cavities had formed. Atypical proliferation of the epithelium of the bronchioles was common. In the lung adjacent to the mediastinal mass, atypical islands of pseudosquamous epithelium with irregular hyperchromatic nuclei resembling those of neoplastic cells were present. The nuclei were large and showed variation in size but no mitotic activity. Some of the pulmonary arterioles were narrowed by a focal eccentric thickening of their walls.

Mediastinal Mass and Biopsied Lymph Node: The lymph node taken from the supraclavicular region two and one-half years prior to death showed a complete absence of normal follicles and many cells with large hyperchromatic nuclei, some resembling Dorothy Reed giant cells somewhat. The picture was consistent with a lymphoma, but a specific diagnosis was doubtful. Superior to the root of the right lung and attached firmly to the right apex was a hard mediastinal mass measuring 5×4×3 cm. (Fig. 1). On section this mass was found to consist of fibrous tissue with yellow-to-brown areas, some of which were calcified. Incorporated in this were reddish-brown spongy lymph node-like structures, the largest measuring 2×1.5×1 cm. Some of these structures were completely necrotic and soft. Clearly defined lymph nodes at the bifurcation of the trachea were anthracotic but not enlarged. A lymph node anterior to the trachea above the bifurcation, measuring 1×0.5×0.5 cm. on section, was found to be composed of soft semisolid homogeneous material. Cultures made from these nodes were sterile. No obviously tumor tissue was found.

Histologically the mediastinal mass consisted chiefly of dense collagenous fibrous tissue, with calcification and necrosis. There were collections of macrophages containing iron and sheets of large cells resembling reticulum cells. Lymph nodes embedded in the mass showed complete disappearance of lymphoid follicles, leaving small collections of lymphocytes, dilated blood vessels, and lymphatics; hyaline bands of collagen; markedly thickened and hyalinized blood vessels; sheets of reticulum cells, and a few lymphocytes. No trace of the previously diagnosed malignant lymphoma was seen. The grossly recognized lymph node situated anterior to the trachea was completely necrotic; only shadows of the original structure were visible.

Aorta: The arch of the aorta was smooth up to the beginning of its transverse segment. At this point on the inferior wall there was an abrupt change in the aortic structure, characterized by

Fig. 4.—Segment of the thoracic portion of the aorta exposed to irradiation. The terminal portion of the arch (above) was unexposed and is separated from the irradiated segment by a rather sharply defined border. At several points fresh but organizing thrombi overlie the thick wrinkled fibrous intima. See Figures 5, 6, and 7 for details of structure of the several layers of the vessel wall.

considerable thickening of the wall as a whole, in places up to 3 mm. Especially noteworthy was the alteration of the intima, which, because of its wrinkled tree-bark-like surface, resembled in striking fashion what we have always associated with syphilitic aortitis (Fig. 4). This striking deviation from the normal extended from a point 1.5 cm. below the orifice of the left subclavian longitudinally along the thoracic aorta for 10 cm., where it abruptly gave way to normal aortic structure. Above and below the lesion the aorta presented a relatively normal appearance, its full thickness being only 1 mm. The only structural change present consisted of narrow streaks of atherosclerosis situated in the abdominal segment.

Multiple sections were chosen at all levels of the aorta for study. Above and below the grossly scarred area the aortic wall was normal. At the junction of the normal and the scarred area there was an abrupt change due to thickening of the wall of the vessel, which was greatest in the intima, subintima, and adventitia. In some areas fibrin was deposited in the fibrous intima and subintima (Fig. 7). Attached to the intima were organizing thrombi projecting as polypoid masses into the lumen. The media of the aorta had undergone almost total necrosis (Fig. 5). In some areas both the intima and subintima were involved in this necrotizing process. In the outer half of the less-damaged media and in the adventitia small collections of lymphocytes were present, chiefly around the vasa vasorum (Fig. 5). As revealed by appropriate staining techniques, the elastic tissue in the necrotic media was extraordinarily well preserved, though in places it was fragmented (Fig. 6). There was some increase in collagen between the elastic fibers, especially in relation to the infiltrate of lymphocytes in the outer part of media. The adventitial thickening was due to dense newly formed collagen in which were incorporated the small nutrient arteries with almost completely obliterated lumens.

Bone Marrow: The bone marrow of the sternum showed a complete absence of all cellular elements, leaving only fat. The marrow of the ribs and the vertebrae showed the usual hematopoietic activity. These findings were confirmed by histologic study.

Other Organs: In none of the organs other than those described in the preceding paragraphs were there alterations of consequence pertinent to the problem of primary interest in this case—irradiation injury. Lesions of incidental interest which were found are included in the anatomical diagnosis.

Microbiologic Studies.—Culture for bacteria and fungi made from the heart blood, necrotic mediastinal nodes, and urine all yielded no organisms.

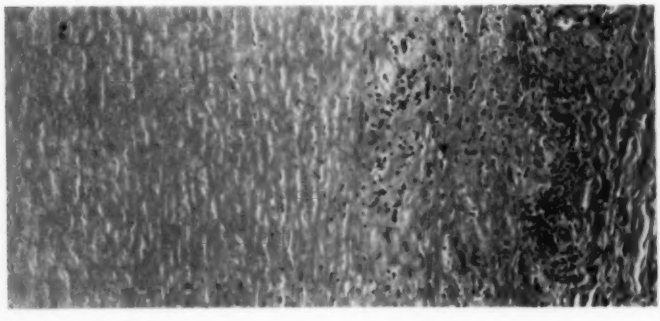

Fig. 5.—Details of the irradiation effects upon the adventitia (right) and the media (left) of the aorta. Swelling and necrosis of the media and fibrosis and round-cell infiltration of the adventitia characterize the lesion.

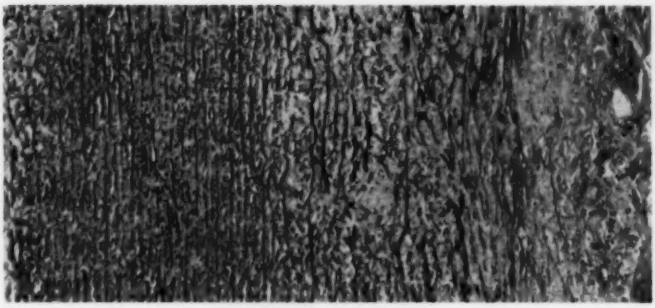

Fig. 6.—The media (left) and the adventitia (right) of the irradiated aorta, stained to define the elastic tissue laminae. Note the persistence of the elastic tissue, although some fragmentation has occurred. Hematoxylin and eosin stain of this section presented a picture much like that in Figure 5.

Stained smears of the materials likewise showed no organism.

Comment

Critical study of all the observations recorded in the case just described allows no doubt that the patient died as the result of injury to vital thoracic viscera, produced by irradiation directed specifically toward the mediastinum for eradication of a lymphomatous process the specific diagnosis of which was uncertain. He received 1,860 r (tumor) units of irradiation during one course of deep x-ray therapy two and one-half years prior to death, an unknown amount one year prior to death, and 100 r units three months prior to death. Although the record does not specify accurately the total amount of irradiation to which this man was subjected, the indicated exposure may be accepted as the minimal amount.

It is well known that the lung receives a considerable amount of radiant energy in the x-ray treatment of mediastinal tumors. Warren and Spencer [1] described alveolar hyaline-membrane formation as one of the characteristics of the pulmonary lesion discovered in cases which had been treated for carcinoma of the breast, esophagus, or bronchus. The interval between the last irradiation and death in their cases varied from one day to two and one-half years. Warren and Gates,[2] in a review of 1,000 consecutive autopsies, noted the alveolar hyaline membrane in five cases in all of which, it was learned later, the chest had been irradiated. They further described the lungs in these cases as rubbery and noncrepitant and attributed this to the flooding of the lung by a homogeneous glassy proteinaceous nonfibrinous exudate. They suggested that irradiation injury to the capillary endothelium may have allowed the formation of the exudate, which is different from that of genuine penumonia, and, furthermore, that partial autolysis of fibrin may have accounted for their failure to demonstrate fibrin in the peculiar exudate. Ellinger [3] has suggested that the components of the typical exudate may be derived from disintegrating cells. Cottier [4] noted in experimental irradiation of rats that the fluid in the lung was rich in both protein and fibrin. Liebow, Warren, and DeCoursey [5] observed cellular necrosis and a pink amorphous granular alveolar substance in

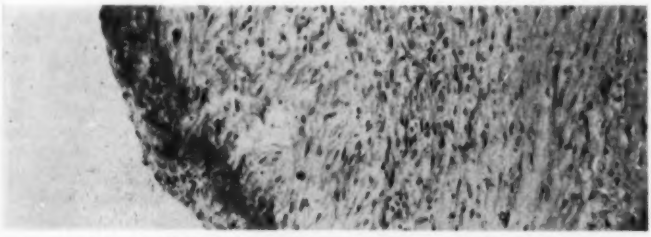

Fig. 7.—The intima of the irradiated aorta. At the left is the intimal surface with a deposit of fibrin which has undergone hyalinization and partial incorporation into the proliferating fibrous tissue. This section represents the whole thickness of the intima at a site corresponding to the sections shown in Figures 5 and 6, the magnification of which is the same.

the lungs of persons who died three weeks after injuries produced by the atomic bombs.

The pulmonary lesion in our own case is identical with that described by others who have studied the problem of pulmonary injury by irradiation. The effects of the radiation upon pulmonary structures are primarily necrotizing and stimulating. Presumably the former precedes the latter, but we have seen nothing in the course of our study of the lung to guarantee that this is so. Nor have we seen anything to convince ourselves that some one component of the lung structure is damaged first—the blood vessels, for example—then followed by consequential changes in other structures. One may reason that this would be the logical sequence, but it is another matter to prove it by what can be seen. All the details of alteration of the pulmonary structure are readily understandable on the basis of what everyone knows about lethal and sublethal injury to tissues and the repair of the damage produced. There is, therefore, no mystery about the irradiation pulmonary lesion, though, to be sure, it is an interesting subject of study. The most intriguing aspect of the problem is the nature of the alveolar exudate, which, as others have pointed out before us, is peculiar. It occurs to us that what we have seen in the alveoli may find its explanation in an alteration of the physicochemical structure of normal blood proteins by the direct action of radiant energy upon these extravasated substances. If this be so, it is a matter of far-reaching significance and deserves extended experimental study.

Reports of small-vessel changes in irradiated tissues record some lesions which are similar to those observed microscopically in the aorta in our case. Woolbach, as quoted by Warren,[6] noted that the endothelium may proliferate to the point of obliteration of the lumen. Windholz[7] described whole intimal proliferation with partial obliteration of the lumen in arteries and complete obliteration in veins. In deep vessels of irradiated skin, Warren[8] found endothelial proliferation leading to occlusion and sometimes necrosis of endothelial cells followed by thrombosis. Sheehan,[9] in a study of small arteries of irradiated organs (rectum, ovaries, and uterus), observed a focal flat thickening of the intima produced by foam cells only, by a combination of fluid and fibrin, or by hyaline material deposited between the endothelium and internal elastic membrane. Such plaques resulted in narrowing or complete occlusion by thrombosis of the vessel. Fibroblastic intimal thickening, hyaline necrosis of media, and infiltration of adventitia by monocytes, lymphocytes, plasma cells, and neutrophils also were described by him. McLaurin et al.,[10] in a study of the effects of gamma and roentgen irradiation of the intact spinal cord of monkeys, observed the vascular effects to be necrosis followed by inflammation and occlusive endothelial proliferation. Thrombosis of the larger pulmonary vessels after irradiation was described by Stone et al.,[11] Fried and Goldberg,[12] and Jacobsen.[13] Bloom[14] noted in experimental animals that the large arteries, including the aorta, showed a brilliant pink staining of the collagenous component immediately after irradiation; after a month this was purplish pink. When very high doses were employed, hyaline necrosis occurred. After strong external β-irradiation the media was transferred into a grossly homogeneous sheath which, nevertheless, under the microscope appeared finely granular. The elastic laminae were highly resistant and, as a rule, did not disappear. In vessels into which radium was injected, the connective tissue cells proliferated, forming uneven plaques which projected into the lumen. Bormann[15] studied the effect of radium emanations inserted into the sinoauricular node of dogs and found fibrinoid necrosis and thrombosis of the larger vessels of the heart; even so there was little change in the character of the elastic tissue. Other workers who studied elastic tissues in irradiation have observed an increase, decrease, or no change in the elastic tissue (Warren[16]).

It will be at once obvious that the details of the pathological processes produced in vessels by irradiation, as briefly reviewed above, are exactly those observed in the aorta in our case. These processes are, however, by no means specific for irradiation. Nevertheless, that they were the effects of irradiation in our case seems assured not only by the circumstances under which the aortic lesion occurred but by the fact that all other possible etiologic mechanisms which might have given rise to the lesion (syphilitic aortitis, for example) can be excluded from consideration.

In our case the extreme situation in both the lungs and the aorta presented no problem in recognition. The problem was one of interpretation. Definitive irradiation injury to the lungs and even to the aorta can hardly be as uncommon as it would appear to be from the infrequent observations on record. The use of radiant energy in its several forms is too widespread and too inadequately controlled to justify one's belief in this scarcity. The actual quantity of radiation received by deep-seated tissues can rarely be accurately determined, and so, with the subtleties of irradiation injury what they are, it is not surprising that only such gargantuan lesions as we have described in our case are recognized. The chronic fibrous interstitial pneumonias and the necrotizing forms of aortitis, so well known and yet so puzzling to us, might well be studied more critically.

Summary

In this paper is reported a study of irradiation injury in a 29-year-old white man who was given three courses of deep x-ray treatment (the total amount of radiant energy used not accurately known) for a lymphomatous tumor of the mediastinum and neck, which had been discovered two and one-half years prior to death. At autopsy the thoracic aorta, both lungs, mediastinal lymph nodes, and sternal bone marrow showed pronounced changes due to irradiation. In both the lungs and the aorta the injury produced was both necrotizing and stimulative. In the other tissues the effects were predominantly necrotizing and suppressive. The lung lesion was a peculiar form of chronic interstitial necrosis, edema, and fibrosis and is very similar to that previously described by other observers. The aortic lesion was a localized necrosis involving all layers of the vessel wall, accompanied by attempts at healing through proliferative changes in the connective tissue. The pulmonary injury was the predominant lethal factor. The aortic lesion, while potentially lethal, resulted in this case in only the minor secondary complications of splenic and renal infarction resulting from dislodged thrombi that had formed at the site of aortic injury. This appears to be the first report of a well-defined lesion in the aorta of man that can be directly attributed to irradiation.

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The Fate of Equine-to-Canine Arterial Heterografts

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Initially, processed homografts were used to bridge vascular defects or to bypass occluded portions of an artery. A supply of homografts could be obtained for replacing parts of the aorta; however, long segments of a peripheral artery suitable for bypass have been more difficult to procure. It was thought that if prolonged maintenance of function could be demonstrated, equine common carotid artery could serve as a bypass connection between peripheral arteries. This artery has no branches requiring ligation and is about the caliber of the femoral artery with comparable width of the wall. Though the use of heterografts proved impractical and homografts have also been replaced by plastic prostheses, the fate of vascular heterografts remains of biologic interest. The present study deals with the structural alterations of the lyophilized equine common carotid artery grafted into the abdominal aorta of dogs.

Method

Fresh carotid arteries were placed in isotonic saline solution. Within six hours after procurement they were immersed in ethylene oxide, then frozen rapidly in an acetone-dry ice mixture, vacuum dried, and sealed in glass containers. Before use, reconstitution was accomplished by immersing the arteries in isotonic saline solution until soft and pliable. Arteries so treated were grafted into the abdominal aortas of 15 mongrel dogs anesthetized by intravenous pentobarbital. The grafts replaced a segment of the aorta about 3 cm. long between the renal arteries and the trifurcation. The anastomoses were performed with continuous 00000 arterial nonabsorbable surgical sutures (silk). On completion of the anastomoses, release of the occluding clamps was followed by minimal bleeding, and the femoral pulses were palpable in every instance. Subsequently, the animals were examined periodically for the condition of the femoral pulses. Of the 15 heterografts, 2 were removed after 15 days; 2, after 60 days, and the rest, after 15 months. The removed specimens were fixed in 10% formalin and after fixation were cut longitudinally; the color was restored by immersion in 80% ethyl alcohol, and they were photographed. Portions from the proximal and distal suture lines and from the center portion were imbedded in paraffin, cut

Fig. 1.—Equine common carotid artery grafted into canine abdominal aorta, 15 days after implantation.

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serially, and stained respectively with hematoxylin and eosin, Masson's trichrome, and Weigert's elastic tissue stains. A normal freshly fixed carotid artery and one lyophilized and reconstituted were similarly imbedded, cut, stained, and studied.

Results

In the two grafts that were in place 15 days, there was good healing along the suture lines and no significant gross change was noted in the walls of the grafts (Fig. 1). In the two heterografts in place for 60 days there was slight dilatation, the lumen being one-fourth more spacious than that of the proximal and distal portions of the aorta. In the graft portion, gray or brown elevated areas were on the intimal surface, the brown areas consisting of a coagulum. The suture lines were glossed over by a thin membrane. There was a slight narrowing of the lumen at the proximal and distal suture lines (Fig. 2*a* and *b*).

Microscopically, in the graft portion the intimal surface was wavy and the subintimal zone loose and fibrillar. In Weigert's preparations the wavy inner surface was bordered by elastic fibers, slightly con-

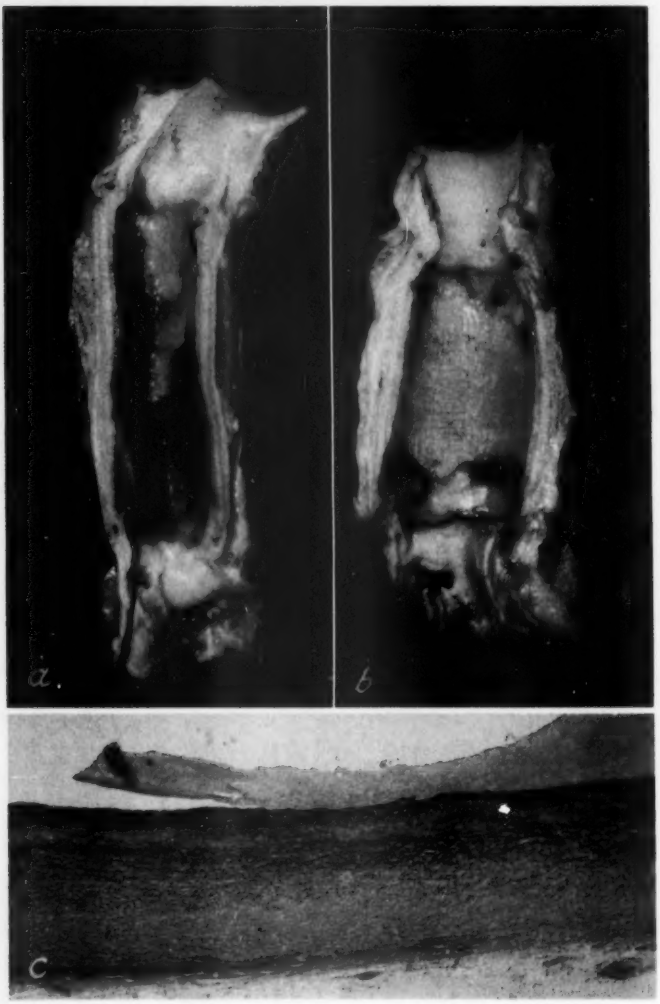

Fig. 2.—Equine common carotid artery grafted into canine abdominal aorta, 60 days after implantation. (*a* and *b*) There is slight dilatation of the graft portion, with some narrowing of the lumen at the proximal and distal suture lines. (*c*) Microscopic appearance of the heterograft. The intimal surface is bordered by elastic fibers slightly condensed. Almost parallel to these are other fibers at varying distances and enclosing light or empty spaces. Newly formed coarse collagenous bundles interspersed with elastic fibers form an outer zone almost as wide as the graft. Weigert's preparation; reduced slightly from mag. × 13.5.

densed. Almost parallel to these were other fibers at varying distances and enclosing light-stained or empty spaces. Newly formed coarse collagenous bundles interspersed with elastic fibers formed a zone almost as wide as the graft. In another zone of similar width no elastic fibers were seen. This was continuous with the adipose tissue of the adventitia (Fig. 2*c*). At the suture line a clot covered the area. Beneath were giant cells of the foreign-body type about the suture material and large mononuclear cells. Capillaries were numerous.

Of the 11 grafts that were in place for 15 months, 1 graft was completely occluded by a thrombus. In the remaining grafts there was aneurysmal dilatation of varying degrees (Fig. 3*a* and *b*). A fusiform enlargement had in some instances almost tripled the diameter of the lumen of the graft. The intimal surface was smooth and covered with a coagulum of varying thick-

Fig. 3.—Aneurysmal dilatation of the equine common carotid artery grafted into canine abdominal artery, 15 months after implantation. (*a* and *b*) The fusiform enlargement almost tripled the diameter of the lumen of the graft. Microscopically, the host tissue completely replaced the graft. (*c*) In this segment of the circumference osteoid trabeculae enclose marrow spaces. Hematoxylin and eosin preparation; reduced slightly from mag. × 13.5.

ness. The suture lines were glossed over by a thin film of tissue. In the graft portion marked sclerotic changes thickened or thinned the wall. The fusiform dilatation resembled aneurysmal dilatation of the human abdominal aorta. No changes occurred in the aorta proximal and distal to the graft.

Microscopically, a zone of clotted blood was attached to the intimal surface. Aggregates of large mononuclear cells with foamy cytoplasm were between delicate endothelium-lined spaces. The rest of the wall was made up of coarse collagenous bundles of varying width. In the adventitia there were numerous blood vessels and nerve bundles in connective tissue. Lobules of adipose tissue cells were adjacent here and there. The host tissue apparently completely replaced the graft. In one segment of the circumference bone formation was noted with osteoid trabeculae enclosing marrow spaces. These contained young connective tissue, capillaries, large foam cells, and occasional osteoblasts (Fig. 3*c*).

In order to evaluate the changes in the heterograft further, the structure of the freshly fixed intact common carotid artery was compared with the lyophilized artery. On transverse sections of the equine carotid artery stained with hematoxylin and eosin the intimal surface was wavy and thrown into low delicate folds with corresponding depressions. The nuclei of the intimal lining were discernible at intervals. They rested on a thin elastic lamina that followed the elevations and depressions of the wavy surface. The media was composed of cells with elongated slightly curved or bent nuclei in a slender cytoplasm with indistinct margins. Wavy elastic fibers were interwoven at distances of two to four cell nuclei. In the adventitia there were many coarse collagenous bundles or elastic fibers. These were wavy and had a circular course. In preparations stained with Masson's trichrome stain (Fig. 4*a*) the appearance was essentially similar, with the cell nuclei of the intimal lining discernible and the elastic fibers inconspicuous. The muscle fibers stained red and the connective tissue stroma blue. Some of the elastic fibers stained a deeper red than the smooth muscle cells. In the adventitia bundles of the blue- and red-stained fibers were interspersed. In preparations stained with Weigert's elastic tissue stain (Fig. 4*b*), the cells of the intima were barely discernible. The delicate elastic lamina outlined the intimal surface. Wavy elastic fibrils stained dark brown, ran parallel at intervals as in the hematoxylin and eosin preparations. Similar coarse brown fibrils were interspersed with the coarse collagenous bundles in the adventitia. In preparations cut longitudinally the endothelial cells of the intimal surface were continuous and following the elevations and depressions of the inner elastic lamina. The cell nuclei of the media ran perpendicular to the lumen and were bordered by connective tissue subdividing them into squares or bundles. In the adventitia

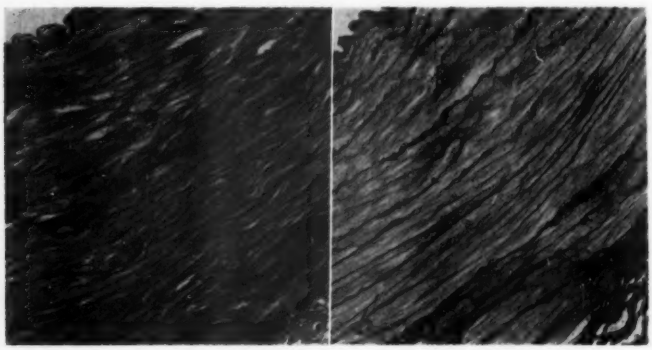

Fig. 4. — Microscopic appearance of the fixed equine common carotid artery in cross section. (*a*) Masson's trichrome preparation; × 75. (*b*) Weigert's elastic tissue stain; reduced 15% from mag. × 75.

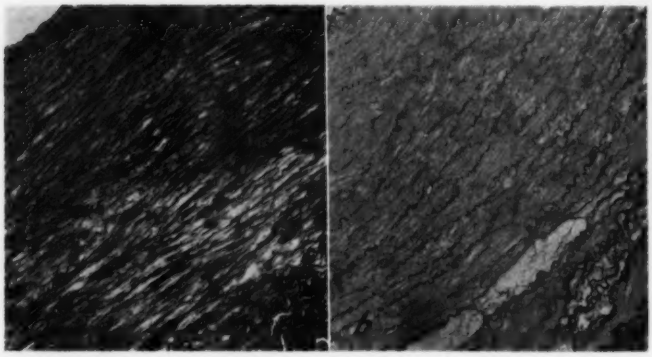

Fig. 5. — Microscopic appearance of the lyophilized equine common carotid artery. (*a*) Masson's trichrome preparation; ×75. (*b*) Weigert's elastic tissue stain; reduced 15% from mag. ×75.

collagenous and elastic fibers ran parallel with the surface. Most of the vasa vasorum were cut transversely or obliquely and were conspicuous. In the Masson's preparations an envelope of blue fibrils surrounded individual smooth muscle fibers and broader septa surrounded the muscle bundles. The blue fibrils were continuous with those of the adjacent adventitia. About twice as many fibrils stained blue than red. In Weigert's preparations the endothelial cells of the intima were not discernible. Brown fibrils formed a network around smooth muscle cells, with elastic fibers at intervals running parallel to the surface. Heavy brown fibrils separated coarse collagenous bundles, which were twice as abundant as the pink-stained fibrils.

On transverse sections of the lyophilized and reconstituted artery, the microscopic structure was essentially similar to that of the freshly fixed equine artery. However, in focal areas there were vacuolation and destruction of the muscle fibers. Slit-like spaces were at irregular intervals and at various distances from the intimal and adventitial surfaces. These changes in the media were more conspicuous and exaggerated in the preparations stained with Masson's trichrome stain (Fig. 5*a*). A coagulum seemed to fill the larger spaces with anuclear protein masses in the coagulum. In Weigert's preparations the course of the elastic fibers appeared slightly modified, and vacuolated spaces corresponded to muscle fibers which had faded or disappeared (Fig. 5*b*). In preparations cut longitudinally and stained with hematoxylin and eosin spaces in approximately the same location were in the media separating the fibrils. The intimal lining was preserved. In the media the change was more marked in the outer one-third than in the inner one-third of the wall. In Masson's preparations the muscle fibers seemed to have dropped out, with small and larger vacuoles replacing them. Weigert's preparations also revealed similar various-sized vacuoles corresponding to dropped-out smooth muscle cells.

Comment

Some investigators have made vascular transplants from the human, the monkey, the cow, the hog, the goat, the cat, and the rabbit into dogs.[1-5] Satisfactory results were reported when the graft was dried or fresh-frozen and irradiated.[6] Most of the successful experiments, however, were terminated in six months—a period that is apparently inadequate for the evaluation of the fate of heterografts. More recent experiments have demonstrated that heterografts failed to maintain function regardless of the method of preparation.[7]

The information gained from our experiments is of considerable interest. An artery with muscular media, such as the equine carotid artery, is seriously damaged by the lyophilizing process. The injury to the muscle fibers weakens the wall; so by 60 days the graft portion bulges, and by the

15th month the entire wall is replaced by host tissue. The latter is made up of dense hyalinizing fibrous connective tissue with retrogressive change—hyalin or progressive bone formation.

Summary

Lyophilized equine common carotid artery was grafted into the canine abdominal aorta. Gross and microscopic studies were made of the grafts removed after 15 and 60 days and 15 months, respectively. Aneurysmal dilatation of the graft portion occurred after 60 days, and complete replacement of the graft by host tissue ensued by the 15th month.

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Immunochemical Demonstration of Fibrin in the Generalized Shwartzman Reaction

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The composition of the "hyaline" thrombi appearing in the capillaries, arterioles, and venules of various organs of rabbits exposed to two intravenous injections of bacterial endotoxin has not been made clear. Circumstantial evidence has for some time indicated that the thrombi were primarily composed of fibrin, derived from the fibrinogen of the circulating blood.[1,2] It is the purpose of this report to describe the demonstration of fibrin in these thrombi by use of the Coons and Kaplan [3] fluorescent-antibody method.

Materials and Methods

The generalized Shwartzman reaction was induced in eight 1 kg. rabbits by the intravenous injection of two doses of 0.2 mg. of Shear's polysaccharide (Serratia marsescens bacterial endotoxin)* 24 hours apart. All the animals developed bilateral renal cortical necrosis. At autopsy sections were taken from liver, kidney, spleen, and lungs and one set fixed in formalin and the other fresh-frozen on dry ice. The formalin-fixed tissues were stained with phosphotungstic acid hematoxylin. The unfixed frozen sections were cut in a cryostat at 6μ at −20 C and subsequently treated by an adaptation to rabbit fibrin of a technique previously described by Gitlin et al.[4] for human fibrin. The sections were placed on glass slides and dried rapidly. The mounted sections were placed in 95% alcohol for 30 seconds, washed for 20 minutes with four changes of 0.15 M NaCl, and then flooded with fluorescein-labeled hamster antiserum against rabbit fibrin. The washing procedure served to wash fibrinogen as well as other soluble proteins from the section, leaving behind the insoluble fibrin.

Alternate sections were stained with labeled antiserum that had been adsorbed either with purified fibrinogen or with washed fibrin prepared from purified fibrinogen solutions. By pretreating the sections with unlabeled hamster antifibrin and washing before applying labeled antiserum, additional staining controls were obtained; in these instances, combination of the unlabeled antibodies with fibrin specifically inhibited the subsequent uptake of labeled antibodies.

All sections were washed after interaction with labeled antisera, by use of four changes of 0.15 M NaCl over a period of 20 to 30 minutes. The sections were mounted in buffered glycerin (glycerol) and examined with an ultraviolet light microscope for the presence of specific yellow-green fluorescence indicating the distribution in the section of the protein being studied.

The material in washed tissue sections reacting specifically with fluorescent antifibrin antibodies is considered to be fibrin, or an insoluble derivative of fibrinogen.

Observations

The hyaline thrombi observed in the phosphotungstic acid hematoxylin preparations in the small vessels of the lungs, spleen, liver, and kidney all exhibited specific yellow-green autofluorescence of the bound labeled antibody, and all other parts of these tissues failed to react. Photomicrographs of thrombi in the liver and kidney stained with phosphotungstic acid hematoxylin along with their immunohistochemical counterparts are presented in Figures 1-6.

With respect to the liver, there is an extensive diffuse deposition of fibrin on the

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From the research laboratory, Department of Pathology, Free Hospital for Women, Brookline, Mass., and the Departments of Pathology, Obstetrics, and Gynecology, Harvard Medical School, Boston (Dr. McKay); from the Children's Medical Center and the Department of Pediatrics, Harvard Medical School, Boston (Drs. Gitlin and Craig).

* Supplied by Dr. Murray J. Shear, of the National Institute of Health.

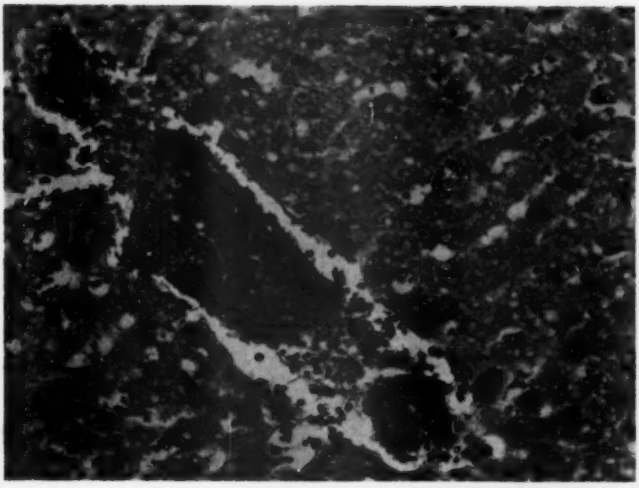

Fig. 1.—Thrombus in hepatic central vein. Phosphotungstic acid hematoxylin; × 200.

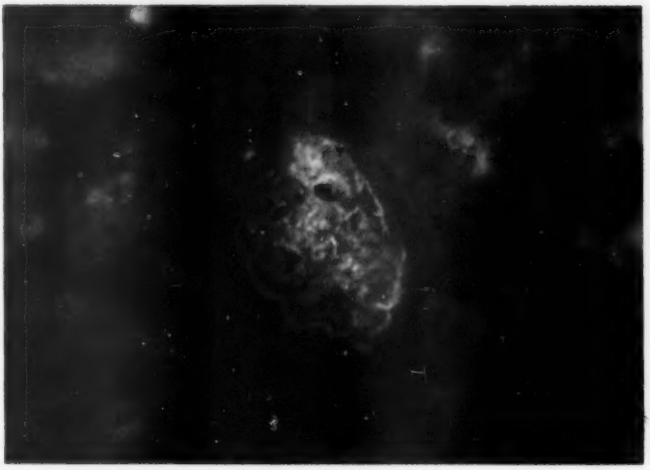

Fig. 2.—Thrombus in hepatic central vein reacted with fluorescein-labeled antifibrin serum and photographed with ultraviolet light. The light areas in the liver cords have a blue rather than green fluoresence, but this distinction is not clear in the black-and-white print; × 210.

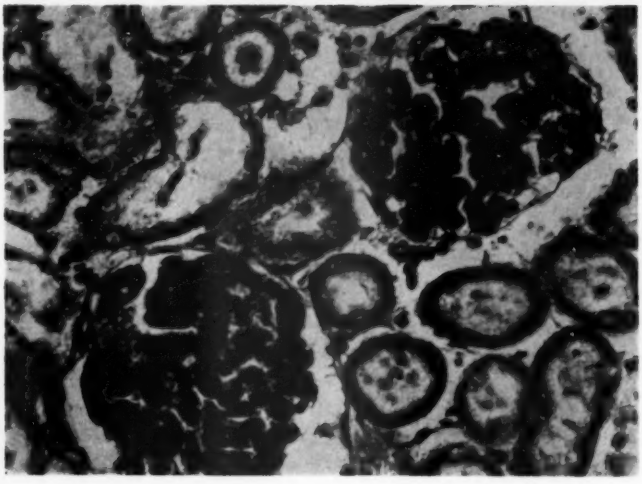

Fig. 3. — Thrombi in renal glomeruli. Phosphotungstic acid hematoxylin; × 400.

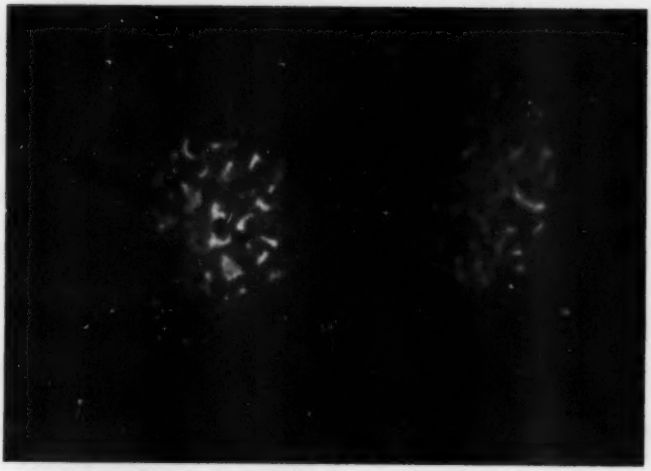

Fig. 4. — Thrombi in renal glomeruli showing specific fluorescence indicating the presence of fibrin. Ultraviolet light; × 210.

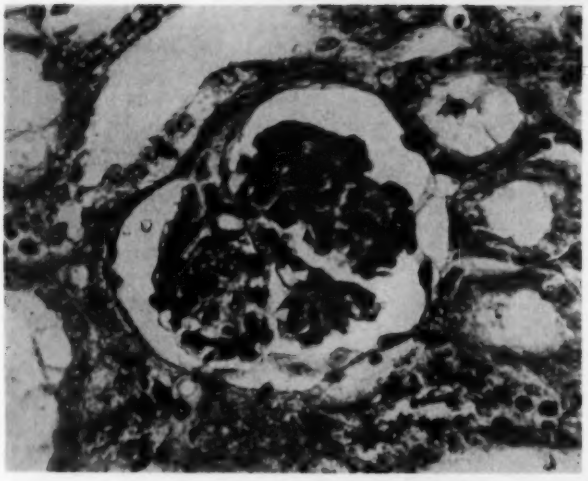

Fig. 5.—Renal glomerular thrombus. Phosphotungstic acid hematoxylin; × 400.

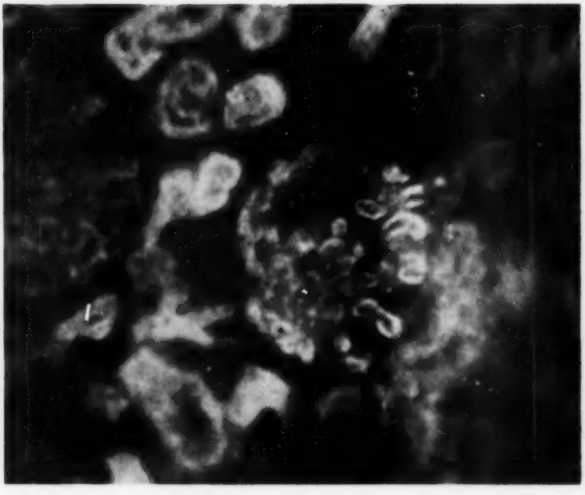

Fig. 6.—The presence of specific antibody fluorescence in the glomerular thrombus indicates the presence of fibrin. The bright necrotic renal tubules surrounding the glomerulus have a blue fluorescence, which is readily distinguished in the original microscopic examination from the yellow-green antibody stain. Ultraviolet light; × 210.

endothelium of the sinuses, found by the immunochemical technique which is not evident in the hematoxylin and eosin or phosphotungstic acid hematoxylin preparations. In the kidney the renal tubules are necrotic as a result of ischemic infarction and show a bright blue fluorescence. The specific antibody yellow-green fluorescence appeared in the kidney only in the thrombi in the arterioles and glomeruli.

Comment

The demonstration of fibrin in the "hyaline" thrombi of the generalized Shwartzman reaction by immunohistochemical means confirms the concept derived from conventional histologic examination and from blood coagulation studies.[1,2] Vazquez and Dixon[5] have also reported the same observation. In addition, Pappas et al.[6] have provided evidence of the presence of fibrin in these thrombi by use of the electron microscope. These three studies leave little doubt as to the basic nature of the thrombi. This does not mean that other substances may not be present in the thrombi. Muirhead et al.[7] have shown the presence of neutral fats, fatty acids, cholesterol and its esters, phospholipids, and glycoprotein in the thrombi. These substances are all present in the circulating blood and are probably simply "occluded" in the fibrin thrombi forming in the blood stream. The prevention of thrombosis after exposure to bacterial endotoxin by use of heparin,[8,9] warfarin sodium,[10] and streptokinase[11,12] indicates the primary significance of the clotting of fibrinogen in the pathogenesis of this reaction.

Summary

By use of the Coons and Kaplan fluorescent-antibody technique it has been demonstrated that fibrin or an insoluble derivative of fibrinogen is a constituent of the thrombi found in the lungs, spleen, liver, and kidney in the generalized Shwartzman reaction. In combination with studies of the blood coagulation mechanism it is concluded that fibrin is the essential constituent of these thrombi.

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Progressive Cellular Alterations of Lymph Nodes

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Attention has been recently called to the frequency with which enlargement of superficial lymph nodes is encountered in a variety of grave and often fatal clinical systemic diseases.[1] Microscopic examination of such enlarged nodes characteristically disclose a hyperplasia in which both lymphocytic and reticuloendothelial cells participate. Plasma cells are often present and frequently have a periodic acid-Schiff-positive cytoplasm. Because lymph node enlargement was often antecedent to the development of other clinically recognizable evidence of disease, it was urged that the clinical course of patients with unexplained or generalized lymphadenopathy be followed closely.

The present report is an extension of the investigation of lymph node changes in a variety of diseases. Particular consideration is given to the progressive alterations in maturation of cells and modifications of their distribution and to certain cytologic and cytochemical changes which occur in the plasma cells.

Materials

Lymph nodes obtained by biopsy and at autopsy were used in this study (Table 1). There was a total of 506 cases. Many of these were represented by several lymph nodes. Thirty-four of the patients, who were not autopsied, had two to four biopsies, with a minimum of one month and a maximum of five years between biopsies. Sixty-four of the patients, who were autopsied, had previous lymph node biopsies. There were an additional 116 autopsy cases, and the remainder were single surgical specimens.

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Methods

The lymph nodes were fixed in 10% formalin, and paraffin sections were prepared. After removal of the paraffin and hydration, the sections were stained with hematoxylin and eosin, the periodic acid-Schiff (PAS) method,[2] toluidine blue,[3] and a combination of PAS and toluidine blue or hematoxylin. Additional sections of the same nodes were treated with α-amylase,[*,4] β-glucuronidase,[*,5] ribonuclease,[*,4] and the combinations of ribonuclease-β-glucuronidase, β-glucuronidase-amylase, ribonuclease-β-glucuronidase-amylase. The PAS procedure and the hematoxylin staining were performed on all enzyme-treated sections. The lymph nodes were examined for their histologic features, the presence of plasma cells, and the tinctorial reactions of the cytoplasm of the plasma cells.

Results

Histology of Lymph Nodes.—Several general histologic patterns were noted in the lymph nodes from this large variety of related and unrelated diseases (Table 1). Lymph nodes obtained when enlargement was first noticed clinically had the following histologic pattern.

The follicles were prominent, with primary centers of small mature lymphocytes and secondary centers of lymphoblasts (Fig. 1). The remainder of the cortex was packed with lymphocytes of varying degrees of maturity (Fig. 2). The sinusoids were dilated and lined with many more reticuloendothelial cells than found in the normal node (Fig. 3). The sinusoids often contained a mixture of macrophages and mature lymphocytes (Fig. 3).

* Nutritional Biochemicals Corporation, Cleveland.

TABLE 1.—*Cases from Which Lymph Nodes Were Obtained*

	Biopsy	Autopsy	Comparison of Average Plasma-Cell Response
Normal lymph nodes			
Trauma (amputations)	15	0	±
Sudden death			
Trauma	0	5	±
Coronary artery disease	0	10	±
Hyperplasia of lymph nodes (Patients living & well)	70	0	1+
Carcinoma			
Lung	25	7	3+
Stomach	10	5	3+
Breast	25	5	3+
Cervix	5	1	2+
Colon	20	3	3+
Esophagus	2	1	2+
Kidney	4	0	2+
Liver	1	0	3+
Lymphoma & leukemia			
Lymphosarcoma	17	12	0
Hodgkin's disease	17	11	4+
Granulocytic leukemia	14	7	2+
Lymphocytic leukemia	1	3	0
Reticulu n-cell sarcoma	1	0	4+
Multiple myeloma	1	7	2+
Benign tumors	3	0	1+
Granuloma			
Tuberculosis	10	0	3+
Sarcoidosis	7	2	2+
Nonspecific	11	0	3+
Diffuse pulmonary fibrosis	4	2	3+
Bronchiectasis	6	0	2+
Cirrhosis	2	13	3+
Polyarteritis	3	22	4+
Disseminated lupus erythematosis	28	26	4+
Rheumatoid arthiritis	4	2	3+
Rheumatic heart disease	2	12	2+
Dermatomyositis	1	2	3+
Scleroderma	0	1	3+
Amyloidosis	0	3	4+
Thrombotic thrombocytopenic purpura	0	2	2+
Asthma	0	2	2+
Glomerulonephritis	0	8	3+
Dermatitis			
Contact	5	0	3+
Drug eruption	2	0	2+
Exfoliation	10	0	4+
Complications of arteriosclerosis	0	4	1+

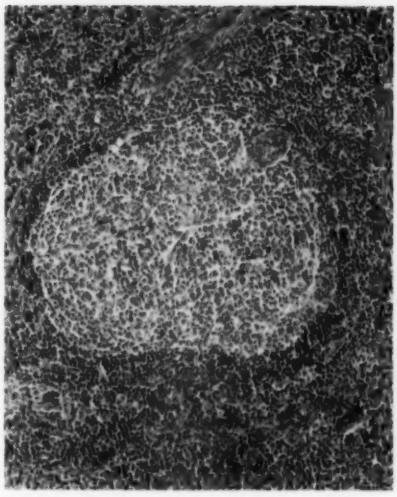
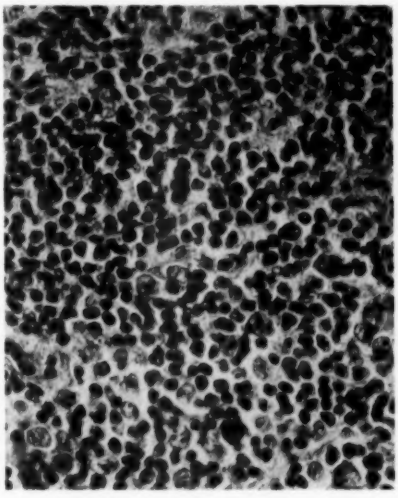


Fig. 1.—Follicle with narrow band of mature lymphocytes and prominent secondary center. Hematoxylin and eosin; × 100.

The plasma cells were more numerous in the medulla than in the normal node. They were located between the sinusoids and intermixed with mature lymphocytes and immature cells of the lymphocytic and/or plasma-cell series (Fig. 4). Plasma cells were not identified in the cortex.

Fig. 2.—Area of cortex away from follicle with lymphocytes of varying maturity. Hematoxylin and eosin; × 450.



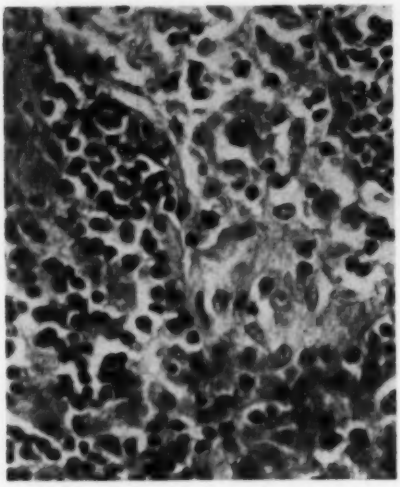


Fig. 3.—Sinusoid with prominent lining cells and with macrophages and lymphocytes in the lumen. Hematoxylin and eosin; × 450.

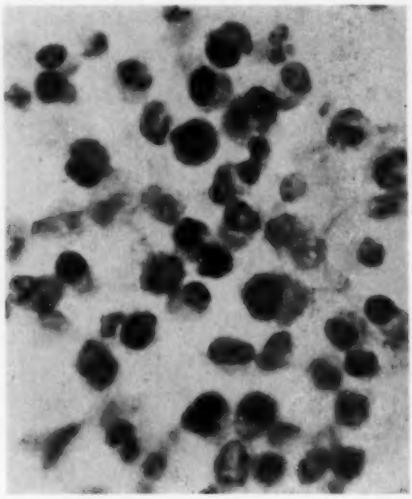


Fig. 4.—Plasma cells, mature lymphocytes, and immature cells of the lymphocytic and/or plasma cell series in the medulla of a lymph node. Hematoxylin and eosin; × 1,200.

Later biopsy specimens from the same patients had a histologic pattern that differed from the early ones. The cellularity of the node was characteristically diminished, and the distinction between cortex and medulla was less prominent or absent (Figs. 5 and 6). The follicles tended to be smaller, few in number, or not discernible (Figs. 5 and 6). The sinusoids were prominent and differed from the preceding group in that there were fewer reticuloendothelial cells (Fig. 7). Frequently the sinusoids contained lymphocytes, macrophages, and plasma cells (Fig. 8). When lymph nodes

Fig. 5.—Partial loss of distinction between cortex and medulla and decrease in number and size of follicles. Hematoxylin and eosin; × 6.

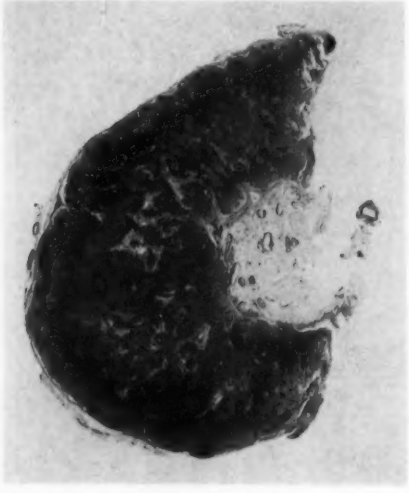
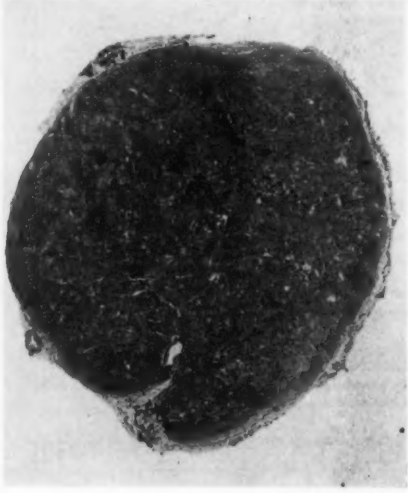


Fig. 6.—Complete loss of distinction between cortex and medulla and absence of follicles. Hematoxylin and eosin; × 6.



were obtained late in the clinical course of a disease or at autopsy, a similar pattern was present.

The plasma-cell population tended to be high in this latter histologic pattern, while the lymphocytic population was diminished (Fig. 9). The plasma cells were present throughout the node and not confined to the medulla, as seen in the earlier nodal response.

Histochemistry.—Based on the histochemical procedures used, plasma cells in these lymph nodes fell into four categories: (1) the plasma cell in the normal node with a moderately basophilic and PAS-positive cytoplasm; (2) intense basophilia and faintly PAS-positive cytoplasm; (3) decreased basophilia and moderately PAS-positive cytoplasm, and (4) acidophilia and intensely PAS-positive cytoplasm (Table 2).

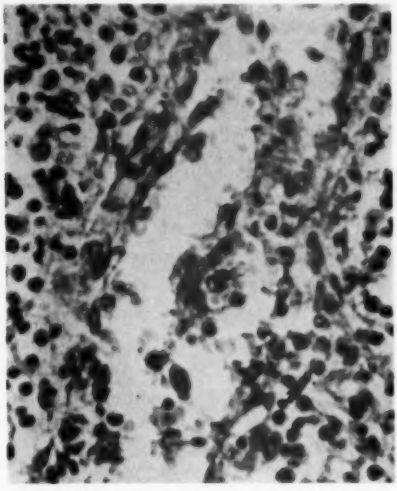


Fig. 7.—Dilated sinusoid with less prominent reticuloendothelial cells. Compare with Figure 3. Hematoxylin and eosin; × 450.

The histochemical properties and the effects of the various enzymes used are shown in Table 3.

Several important observations are apparent from Table 3. α-Amylase had no effect on the PAS reaction of the plasma cells. β-Glucuronidase always reduced the intensity of the PAS-positive response and the acidophilia of the cytoplasm, when present (Fig. 10*A* and *B*). After digestion with β-glucuronidase, basophilia could be discerned in the cytoplasm of the plasma cells of Category 4. Cytoplasmic basophilia was absent in all cases after removal of ribonucleic acid by ribonuclease (Fig. 10).

When basophilic and PAS tests were done simultaneously the reactions were in

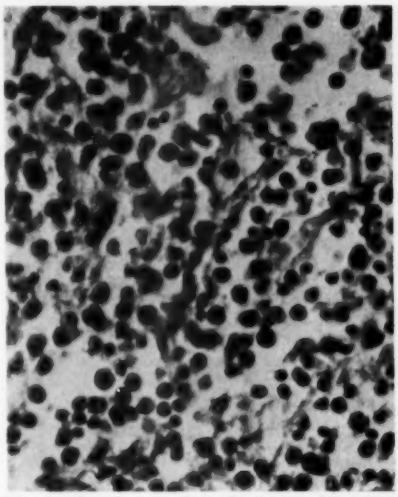


Fig. 8.—Portion of sinusoid filled with lymphocytes and plasma cells. Hematoxylin and eosin; × 450.

Fig. 9.—Decrease in lymphocytes and increase in plasma cells in lymph node associated with chronic disease. Hematoxylin and eosin; × 400.

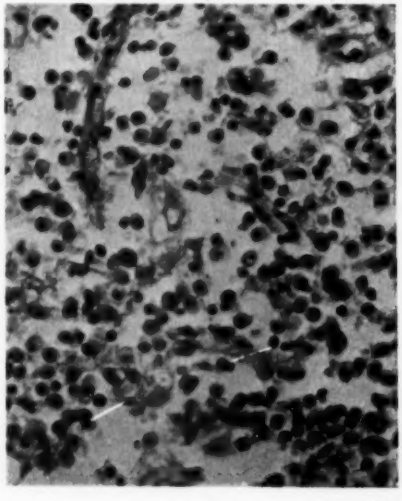


TABLE 2.—*Distribution of Histochemically Different Types of Plasma Cells in the Various Lymph Node Patterns*

	Plasma-Cell Categories			
	1	2	3	4
Normal lymph nodes	Few	None	None	None
Lymph nodes with prominent follicular pattern	Few	Predominant	Few	Rare
Lymph nodes with loss of follicular pattern	None	Few	Occasional	Predominant

TABLE 3.—*Histochemical Behavior of the Plasma Cell*

	Plasma-Cell Categories			
	1	2	3	4
Histochemical Properties *				
Basophilia	2+	4+	1+	0
Basophilia after α-amylase	2+	4+	1+	0
Basophilia after β-glucuronidase	2+	4+	1+	1+
Basophilia after ribonuclease	0	0	0	0
PAS reaction	0-1+	1+	2-3+	4+
PAS after α-amylase	0-1+	1+	2-3+	4+
PAS after β-glucuronidase	±	±-1+	1-2+	1-2+
PAS after ribonuclease	1+	2+	3+	4+
Acidophilia	0	0	0	0
Acidophilia after β-glucuronidase	0	0	0	Reduced

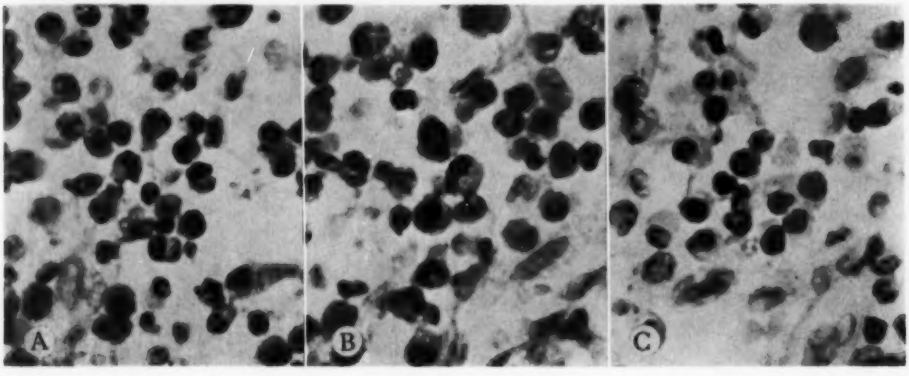
* The combinations of enzymes gave the cumulative effect of each enzyme used separately.

different regions of the cytoplasm. In those cells with intense cytoplasmic basophilia there was a PAS-positive focus adjacent to the nucleus. When the PAS reaction was predominant the cytoplasm was pink to red, with basophilic foci.

Comment

A progressive alteration in the histologic pattern of lymph nodes is frequently associated with a variety of unrelated disease. The initial change consists of enlargement of follicles with prominent germinal centers, increase in number and immaturity of lymphocytes throughout the cortex and medulla, prominent sinusoids with numerous lining cells, and more frequent plasma cells in the medulla. This pattern is apparently altered if the disease process continues. There is a loss of distinction between cortex and medulla, a disappearance of follicles, a decrease in mature lymphocytes, and an increase in plasma cells. Immature lymphocytes and/or plasma cells are sparsely scattered throughout the node. The sinusoids are dilated and frequently contain macrophages, lymphocytes, and plasma cells. There is a variability in the number of reticuloendothelial cells lining the sinus-

Fig. 10.—This is a diagrammatic representation of the effect of the enzymes. The plasma cells are in the same general area of serial sections from a lymph node. *A*, periodic acid-Schiff-positive cytoplasm of plasma cells. *B*, cells after β-glucuronidase. *C*, cells after treatment with β-glucuronidase and ribonuclease. Periodic acid Schiff and hematoxylin; reduced 25% from mag. × 1,200.



oids, depending upon the duration of the disease.

In contrast to the normal nodes (Table 1), the affected nodes contain numerous mature plasma cells with different histochemical properties. The increased number and location of plasma cells, along with the transition from basophilic to acidophilic and PAS-positive cytoplasm, seems to reflect the severity of the disease and its duration.

In lymph nodes which had a prominent follicular pattern and an increased cellularity the majority of the plasma cells had a more intense cytoplasmic basophilia than those of the normal node. Occasional cells were present with an acidophilic and moderately PAS-positive cytoplasm. This is in contrast to the basophilic and weakly PAS-positive cytoplasm of the plasma cells found in normal nodes. Lymph nodes which had a loss of distinction between cortex and medulla, no follicles, and an over-all decrease in cellularity had a large number of plasma cells with acidophilic and strongly PAS-positive cytoplasm.

The failure of α-amylase to alter the PAS reaction excludes the accumulation of intracytoplasmic glycogen. Part of the PAS-positive material is a substrate for β-glucuronidase and is found in all plasma cells. It is more abundant in those cells with acidophilic cytoplasm and represents a portion of the acidophilic cytoplasmic material. It is apparently a glucuronide of some kind. The production of β-glucuronidase substrate is a normal function of the plasma cell. The large amounts found in the altered plasma cell may be related to the general defense processes of the host, perhaps to the detoxication mechanism by glucuronide conjugation.

The PAS-positive material which remains after digestion with β-glucuronidase may very likely fall into the class of glyco- or glucoprotein, as suggested by Pearse,[6] White,[7] and Bessis.[8]

The acidophilia in plasma cells does not indicate a disappearance of cytoplasmic ribonucleic acid. When much of the PAS-positive material is removed by β-glucuronidase, the basophilia due to ribonucleic acid is apparent. The amount of PAS-reactive material is always greater in the affected nodes and is a progressive function of stimulation of the lymph node.

Considerable evidence has been presented implicating a plasma-cell response to a variety of experimental stimuli. Pituitary growth hormone,[9,10] generalized stress,[9-11] and immune reactions,[12-14] among others, have been considered. In each of these experimental conditions some or all of the histologic and cytologic responses reported here have been described. These are follicular enlargement with prominent germinal centers, increase in immature cells in cortex and medulla, and an increase in plasma cells in the medulla.[12-14] The plasma cells in such experimental conditions have a large amount of cytoplasmic ribonucleic acid.[15]

The marked plasma-cell response associated with the variety of disease processes reported here makes an assignment of a specific stimulus uncertain, although the response appears to be similar in all cases. From these findings it would appear that the plasma cell is a multifunctioning cell which responds to a variety of stimuli.

Summary

There is a fairly characteristic histologic pattern of lymph nodes associated with a variety of diseases. The cytologic and cytochemical modifications of the plasma cells in these nodes are described. When the disease state is prolonged there is an increase in periodic acid-Schiff-positive material in the cytoplasm of these cells, a portion of which is a substrate for β-glucuronidase.

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Histochemical Study of Aminopeptidase in Metaplasia and Carcinoma of the Stomach

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In the gastric mucosa of man, there is frequently encountered a lesion, termed intestinal metaplasia or intestinalization, in which the gastric epithelium is replaced by an epithelium having the characteristics of that of the small intestine. Intestinal metaplasia is often found in the gastric mucosa of the aged. It occurs with very high frequency in stomachs in which gastric carcinomas are present and in addition may also be found in association with a variety of other intrinsic lesions of the gastric mucosa.[1] The great frequency of association of gastric carcinoma and intestinal metaplasia in the same stomach has stimulated the performance of a considerable number of studies bearing on the relationship between the two lesions, but at the present time the precise nature of this relationship has not been established and remains the subject of much disagreement.[1-6]

Recently reported histochemical data pertaining to human gastrointestinal tissues have shown that high activities of the enzyme aminopeptidase occur in the epithelium of the mucosa of the small bowel, whereas, if present at all, extremely low activities of the enzyme exist in the epithelium of the gastric mucosa.[7] This distribution of aminopeptidase activity suggested the possibility that a study of the enzyme in intestinal metaplasia might lead to the demonstration of an enzymatic counterpart to the morphologic metaplasia. If such were the case, as would be indicated by a strongly positive reaction for aminopeptidase in intestinalized gastric mucosa, an additional tool would be available for investigating the relationship between intestinal epithelium, intestinal metaplasia, and carcinoma of the stomach.

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From the Department of Pathology, University of Minnesota Medical School.

This study was aided by a grant from the American Cancer Society and was performed during the author's tenure as a Lederle Medical Faculty Awardee.

Materials and Methods

Tissues Employed.—The present study is based on findings obtained in surgically resected specimens. These include 20 stomachs containing carcinomas and 12 stomachs from patients not having a gastric neoplasm. Of the latter group of specimens, six were from patients with benign gastric ulcers and six from patients with benign duodenal ulcers. Nine specimens of small bowel were obtained. Four of these were removed as part of gastrectomy procedures, and five were removed as part of operative procedures primarily directed at resection of segments of small or large bowel. These include two cases of carcinoma of the cecum, two cases with metastatic nodules in the wall of the small bowel, and one case of chronic ulcerative colitis.

Preparation of Sections.—All of the histochemical studies were performed on 6μ frozen sections cut in a cryostat according to procedures previously described.[8] The sections were fixed in acetone at 4 C from 1 to 24 hours. After fixation, the sections were washed for 10 minutes in isotonic saline and then placed in the reaction mixture.

Materials.—The following are sources of the chemicals employed: *l*-leucyl-β-naphthylamide and tris (tris[hydroxymethyl]aminomethane) (Dajac Laboratories), Garnet G. B. C. (diazo salt of aminoazotoluene) (Imperial Chemical Industries), cysteine · HCl (Schwarz Laboratories, Inc.), and inorganic salts (Mallinckrodt Chemical Works); *dl*-alanyl-β-naphthylamide was made available to us by Dr. M. S. Burstone.

Histochemical Procedures.—Two substrates, *l*-leucyl-β-naphthylamide and *dl*-alanyl-β-naphthylamide, were employed for the demonstration of aminopeptidase. The reaction mixtures were buffered with a tris buffer at pH 7.1 and the sections

were incubated for 7½ minutes at 37 C.[7] A short incubation period was used because diffusion artifacts of the methods render of questionable significance any positive reactions which might be observable only after prolonged incubation.[9]

With the procedures employed, the presence of aminopeptidase activity was indicated by the deposition of a red azo dye. The color depth and azo dye localization obtained with the two substrates were very similar.

Results

Aminopeptidase Activity in Gastric Epithelium.—In none of the surgically resected specimens of stomach was aminopeptidase activity observed in normal-appearing epithelium of the mucosa of any portion of the stomach with either substrate. Likewise, in areas of inflammation a negative reaction was found except where intestinalization had occurred. In some specimens, a very weakly positive reaction was seen in mononuclear cells of the interstitial tissue beneath the surface epithelium.

Efforts to Activate Aminopeptidase in Gastric Mucosa.—Efforts to activate a possibly inactive form of aminopeptidase were carried out by two means. The first was to allow autolysis of gastric epithelium by placing blocks of this tissue in a moist environment at room temperature for periods of 30 minutes, 1 hour, 3 hours, 5 hours, and 21 hours. The second was by the use of potential chemical activators. These included $MgSO_4$, $MnCl_2$, and NaCN, which were each added to the reaction mixture in a 0.001 M concentration.[7,10] An attempt at activation with cysteine was also carried out. This compound causes a precipitate in the reaction mixture. It was, therefore, dissolved in a saline solution into which the sections were placed for 10 minutes immediately prior to incubation. Both 0.001 M cysteine and 0.003 M cysteine, plus 0.001 M $MnCl_2$, were employed in this manner.[11] Neither autolysis nor potential chemical activators resulted in detectable aminopeptidase activity in normal-appearing gastric epithelium.

Aminopeptidase Activity in Epithelium of Small Intestine.—In contrast to the negative reaction of gastric epithelium for

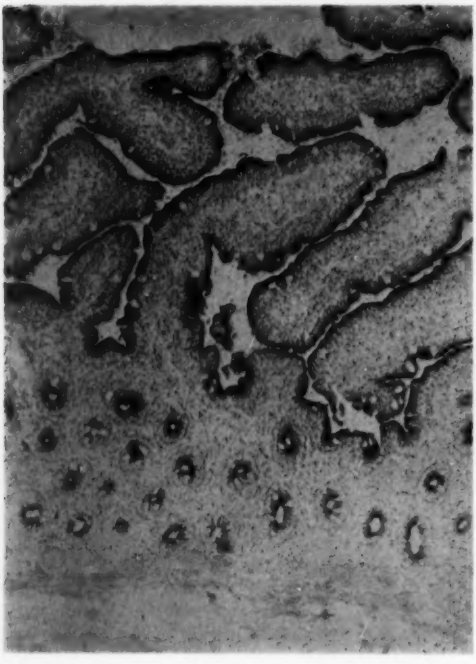

Fig. 1.—Small bowel mucosa. Positive reaction for aminopeptidase appears black and is almost entirely confined to the luminal border of the epithelium. L-leucyl-β-naphthylamide—Garnet G. B. C. procedure; light hematoxylin counterstain; × 65.

Fig. 2.—Intestinal metaplasia of the gastric mucosa. Positive reaction for aminopeptidase is almost entirely confined to the luminal border of the epithelium. Same procedure as Figure 1; × 65.

aminopeptidase, an intensely positive reaction for this enzyme was found in the epithelial cells of the mucosa of all specimens of small bowel studied. This intensely positive reaction was obtained with both substrates and was manifested as a bright red

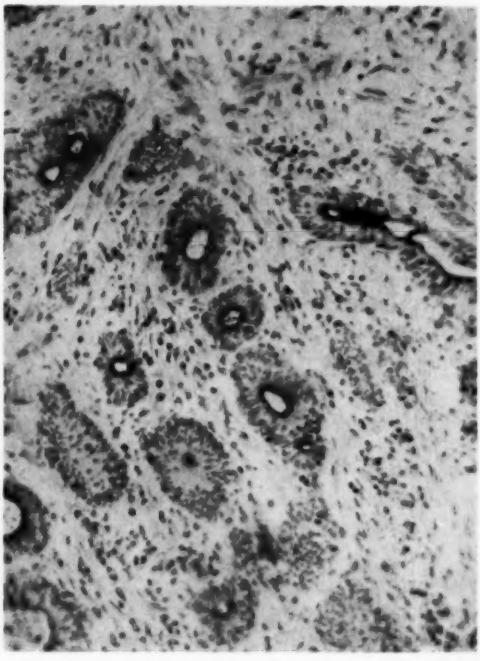

Fig. 3.—Well-differentiated carcinoma of the stomach. Positive reaction for aminopeptidase is almost entirely confined to the luminal border of the malignant glands. Same procedure as Figure 1; × 160.

color located predominantly at the luminal border of the epithelial cells (Fig. 1).

Aminopeptidase Activity in Intestinal Metaplasia of Gastric Mucosa.—The epithelium in intestinal metaplasia of the gastric mucosa gave an intensely positive reaction for aminopeptidase with both substrates. The azo dye was localized predominantly at the luminal border of these cells (Fig. 2). In both intensity of reaction and localization of the deposited azo dye the findings in intestinalized gastric mucosa were very similar to those in the small bowel mucosa.

Aminopeptidase Activity in Carcinomas of Stomach.—In 7 of the 20 carcinomas of the stomach, malignant cells giving an intensely positive reaction for aminopeptidase with both substrates were found. In three other specimens weaker positive reactions were present. The proportion of tumor cells showing a positive reaction varied in different tumors. In some, a small number of cells were intensely positive, and in others, the majority of the tumor cells reacted in this manner. In carcinomas in which well-differentiated glands were present, the deposited azo dye was frequently localized predominantly at the luminal border (Fig. 3), corresponding in this regard to the pattern of azo dye deposition in both normal small bowel epithelium and intestinal metaplasia of the gastric mucosa. In poorly differentiated carcinoma cells, the azo dye generally was diffusely distributed throughout the cytoplasm of the positively reacting cells (Figs. 4 and 5). In some instances, however, only a small segment of the cytoplasm showed a positive reaction. In four specimens, tumor cells showing intensely positive reactions were observed in lymphatics (Fig. 5).

There was no apparent correlation between cellular morphology or the degree of cellular differentiation and the presence or absence of aminopeptidase activity. Likewise, there was no apparent correlation between the particular location of cells within a tumor and the activity of the enzyme. Tumor cells at the periphery of carcinomas, tumor cells in central areas of these lesions, isolated clumps of tumor cells, and tumor

Fig. 4.—Poorly differentiated gastric carcinoma cells infiltrating the gastric mucosa. Tumor cells appear black owing to an intensely positive reaction for aminopeptidase; gastric glands are negative. Same procedure as Figure 1; × 160.

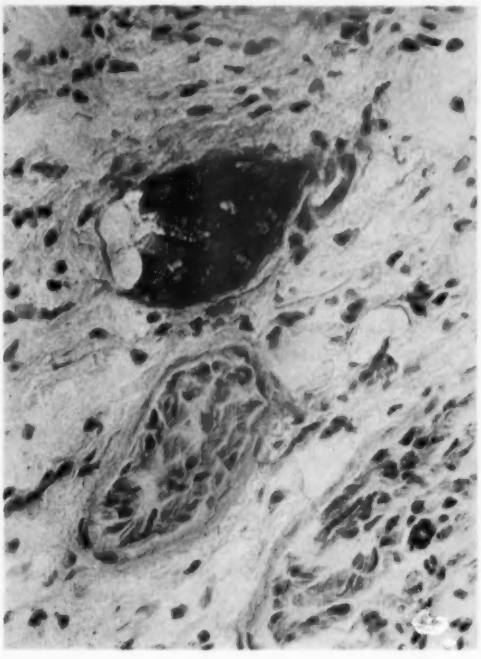

Fig. 5.—Poorly differentiated gastric carcinoma cells in a lymphatic (upper central portion of field). Note intense staining for aminopeptidase. Same procedure as Figure 1; × 400.

cells in lymphatics all have been observed to give positive reactions. There was no indication that inflammation resulted in a positive reaction for aminopeptidase in tumor glands within such areas. In many sections in which both acute and chronic inflammatory infiltrates were seen, a completely negative reaction for aminopeptidase occurred in the tumor glands surrounded by the inflammatory cells.

In some specimens, the mucosa adjacent to the carcinoma was intestinalized and both intestinalized mucosa and carcinoma gave a positive reaction for aminopeptidase. In others, normal-appearing and aminopeptidase-negative epithelium occurred at the borders of the tumor. The presence or absence of a positive reaction for aminopeptidase in a carcinoma did not appear to be related to the amount of intestinal metaplasia observed in the same specimen. Thus, in some instances a carcinoma in a mucosa with large areas of intestinalization would give a negative reaction, whereas some carcinomas occurring in mucosa which showed little metaplasia gave a positive reaction.

Comment

In the present study intense aminopeptidase activity has been found in intestinal epithelium, intestinalized gastric epithelium, and in some gastric carcinoma cells. These findings may be of importance in a number of aspects of the problem of carcinoma of the stomach. The demonstration of an enzymatic relationship between small bowel epithelium and some gastric carcinoma cells suggests that other manifestations of small intestinal metabolic characteristics may be present in these malignant cells, a possibility which could have considerable meaning to biochemical or chemotherapeutic studies of these neoplasms. A second area in which the histochemical demonstration of aminopeptidase may be significant is in better defining the biological behavior of different types of gastric carcinoma cells. The fact that two groups of enzymatically different tumor cells occur in these malignancies presents

a marker to which other distinctive properties, if they exist, may be related. It is of some interest in this regard that tumor cells of very different morphologic appearances give a positive reaction for aminopeptidase. This enzymatic marker is therefore capable of demonstrating a relationship between tumor cells which might otherwise be difficult to perceive.

The occurrence of aminopeptidase activity in intestinal metaplasia and in some gastric carcinoma cells is a finding which would suggest that the positively reacting cells of these two pathologic entities are related. This possibility is further enhanced by the similarity of the intracellular distribution of the enzyme in well-differentiated malignant glands and in intestinalized gastric epithelium. These data would appear to support the much-debated concept that intestinal metaplasia is a precursor to malignancy.[1-6] The point can be raised, however, that carcinoma cells, like normal gastric epithelium, may retain the potentiality of metaplasia and that the acquisition of metaplastic properties, including a positive reaction for aminopeptidase activity, occurs subsequent to malignancy rather than in the cells from which the malignancy originated. On the basis of the present investigation, the two possible sequences of events cannot be distinguished.

Summary

A morphologic histochemical study of the enzyme aminopeptidase has been carried out in gastric mucosa, small bowel mucosa, intestinal metaplasia of the gastric mucosa, and gastric carcinoma.

High aminopeptidase activity has been found in the epithelium of the mucosa of the small intestine, in intestinal metaplasia of the stomach, and in tumor cells of approximately one-third of gastric carcinomas, but no aminopeptidase activity has been observed in the epithelium of normal-appearing gastric mucosa of any portion of the stomach.

In the epithelium of the small bowel mucosa, in intestinal metaplasia, and in many aminopeptidase-positive well-differentiated gastric carcinoma glands, aminopeptidase activity is located predominantly at the periluminal border of the cells.

The high aminopeptidase activity in intestinal metaplasia of the stomach constitutes an enzymatic counterpart to the morphologic metaplasia.

The presence of high aminopeptidase activity in some gastric carcinoma cells is evidence which suggests a metabolic relationship between these cells and those of both intestinal metaplasia and small intestinal epithelium.

Mrs. Delores Thompson provided technical assistance.

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Changes in Desquamated Cells in Carcinogenesis

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Ever since the discovery of the carcinogenic properties of crude tar there have been numerous attempts to induce carcinoma in the uterine cervix of the experimental animal. Although a variety of induction procedures has been investigated over the past three decades, only in recent years have satisfactory induction procedures been evolved. These provide a more or less continuous application of carcinogen to the target tissue and are associated with a higher yield of cancer.[1,2,4,6,8] Through the use of these methods it is possible to study the sequence of changes in experimental carcinogenesis.

On the basis of histopathological evidence carcinogenesis in the mouse cervix can be subdivided somewhat arbitrarily into several stages. Initially, there is an alteration in the surface epithelium that can be designated as dysplasia.[5,8] The second stage is designated as noninvasive cancer by von Haam and Scarpelli, although in our own studies it is designated as early epithelial infiltration,[5] and the lesions are not unlike those classified as pseudoepitheliomatous hyperplasia. This is followed by the development of a squamous-cell cancer, which is usually differentiated and is characterized by the presence of abnormal keratinization.[4,5,8]

The present study deals with the changes in the desquamated cells during carcinogenesis. This detailed analytical study of the cells at various stages in carcinogenesis was carried out in order to determine whether the cellular changes could be correlated with the histopathological alteration. Similarly, since the process of carcinogenesis is dynamic, changes in desquamated cells might provide a useful means for gaining information about the developing lesion without bringing about its destruction.

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From the University Hospitals of Cleveland and the Institute of Pathology, Western Reserve University. U. S. Public Health Service Fellow in Pathology (Dr. Wentz).

Materials and Methods

Female mice of the C$_3$H strain obtained from the Jackson Memorial Laboratory were used. At the onset of the experiment the animals were approximately 16 weeks of age. They were maintained in an air-conditioned room under constant temperature and humidity and given a stock diet with tap water ad libitum.

By use of an induction procedure described by Murphy, a continuous source of carcinogen was provided by means of an impregnated thread. After suitable lengths of No. 12 six-cord cotton thread were washed in changes of water, alcohol, and ether, a knot was formed near the end of each thread by two simple superimposed overhand knots. The knotted ends of the threads were impregnated with 20-methylcholanthrene and yellow wax (beeswax) for an average distance of 3 mm. As determined by weight, each of the threads was impregnated with approximately 1.5 mg. of carcinogen.

The mice were anesthetized by an intraperitoneal injection of 1% pentobarbital sodium. The impregnated thread was inserted into the cervix from below by means of a straight blunt-tipped needle. The needle was first placed in the cervical os. When relaxation was obtained it was passed upward through the right uterine horn. With care to avoid perforating the intestine, the needle was carried outward through the anterior abdominal wall. The thread was anchored in the skin and subcutaneous tissue. The knotted end of the thread was positioned in relation to the uterine cervix.

A cellular sample was obtained by injecting a small amount of isotonic saline into the vagina and aspirating the vaginal contents by means of a capillary pipette. The aspirate was evenly distributed over the surface of a glass slide. Cell films were fixed in 10% buffered formalin and stained by means of the Papanicolaou technique.

Histopathological studies were made on all animals killed. The uterus and upper vagina were removed en bloc and fixed in 10% buffered forma-

lin. The uterus was sectioned in a sagittal plane parallel to the long axis of the organ. In the presence of gross cancer only one microsection was obtained. All other specimens were studied by serial sections, and every fourth section was mounted and stained with hematoxylin and eosin.

Cellular preparations were selected on the basis of the histopathological change which existed and an ample content of cells. A total of 25 normal control specimens was examined. All stages of the normal estrus cycle were equally represented. The cellular sample from the experimental animals was obtained immediately prior to death. Represented are 25 preparations from mice with proven dysplasia, 25 from animals with early epithelial infiltration, and 25 from mice with proven cancer.

The cell films were examined objectively by use of a systematic meander screening pattern. All nucleated epithelial cells in the screening pattern were examined in an attempt to obtain a random sample. A total of 50 cells was studied in each of the 100 preparations. The cell films were projected at 1,760 magnifications by means of a Scopicon,* and the nuclear and cellular outlines were traced on paper by means of a hard-lead pencil. A planimeter was used to compute the nuclear and cellular areas.

TABLE 1.—*Nuclear and Cellular Area* *

	Normal	Dysplasia	Early Infiltration	Invasive Cancer
Cell area, sq. μ	352.75	328.67	292.81	276.76
S. D. †	±94.16	±63.01	±65.84	±40.27
Nuclear area, sq. μ	40.64	92.15	67.87	60.35
S. D. †	±8.50	±13.66	±18.48	±9.87
Relative nuclear area (n/c ratio), %	11.9	28.9	23.2	21.9
S. D. †	±2.42	±5.85	±1.86	±2.92

* Mean nuclear and cellular areas, as determined by planimetry. Values based on a study of 50 cells in each of 25 specimens, or 1,250 cells in each category.

† S. D. represents 1 standard deviation from mean.

Results

As recorded in Table 1, the mean cell area in 25 normal controls was 352.75± 94.16 † square microns. In the presence of dysplasia the mean cell area was 328.67± 63.01 square microns. The mean cell area was 292.81±65.84 square microns in mice with early infiltration and 276.76±40.27 square microns in mice with outspoken cancer.

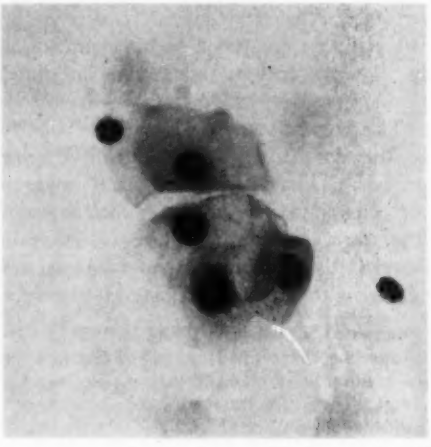
TABLE 2.—*Cellular Morphology* *

Cell Shape	Normal, %	Dysplasia, %	Early Infiltration, %	Invasive Cancer, %
Polyhedral	22.00	30.00	2.32	1.92
Round	35.68	21.76	13.12	11.68
Oval	31.12	33.52	18.32	15.68
Ellipsoidal	7.84	7.76	0.00	0.00
Irregular	3.36	6.88	46.96	31.44
Elongate	0.00	0.00	8.72	15.68
Caudate	0.00	0.08	10.56	23.60
Total	100.00	100.00	100.00	100.00

* Based on a study of 50 cells in each of 25 specimens, or 1,250 cells in each category.

The configuration of the cells is recorded in Table 2. In normal controls, isodiametric forms, including round and polyhedral cells, were relatively common, accounting for 57.68% of the cells. Similarly, in the presence of dysplasia 51.76% of the cells had an isodiametric configuration (Figs. 1 and 2). In contrast, isodiametric forms were less numerous in the presence of early epithelial infiltration and outspoken cancer, accounting for only 15.44% and 13.60% of the cells, respectively. Only 42.32% of

Fig. 1.—Isodiametric cells, including several polygonal and one round form. The nuclei are significantly larger than normal and have an altered hyperchromatic chromatin pattern. There is nuclear vacuolation and a condensation of chromatin beneath the nuclear membrane. Dysplasia; × 500.



* Scopicon, Inc., New York.

† Represents one S. D. from mean.

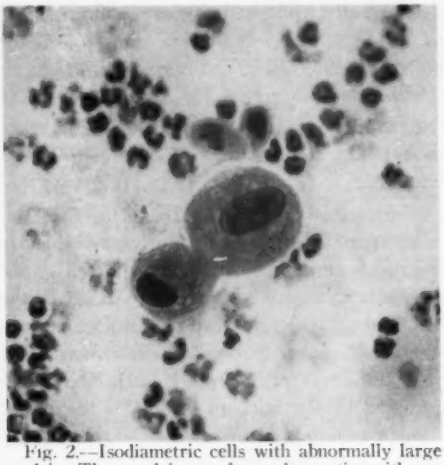


Fig. 2.—Isodiametric cells with abnormally large nuclei. The nuclei are hyperchromatic with an altered chromatin pattern. The nucleus of the larger cell occupies about 25% of the cell area. The cytoplasm is vacuolated and was basophilic. Dysplasia; × 500.

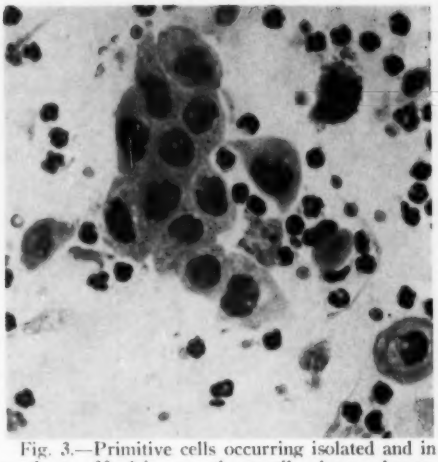


Fig. 3.—Primitive cells occurring isolated and in a sheet. Nuclei are abnormally large, hyperchromatic, variable in shape, and in many instances vacuolated. A rare bizarre cell is observed. Leukocytes and erythrocytes are present. Early infiltration; × 500.

the normal cells had a nonisodiametric configuration, as did 48.24% of the cells observed in the presence of dysplasia. Nonisodiametric forms, including oval, ellipsoidal, irregular, elongate, or caudate cells, were commoner in early infiltration and cancer. They accounted for 84.56% of the cells in early infiltration and 86.40% of the cells from cancer (Figs. 4, 5, and 6). The latter included a greater proportion of elongated and caudate forms as compared with the cells in early infiltration.

Some of the cells encountered in the screening pattern were arranged in groups (Fig. 3), while many were isolated. In the normal, 99.68% of the cells were isolated,

Fig. 4.—Caudate form with irregular hyperchromatic nuclear mass. A bizarre binucleate vacuolated cell is also present. Neutrophils and erythrocytes are represented. Early infiltration; × 500.

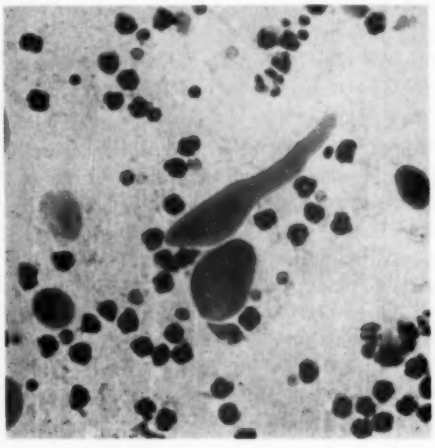
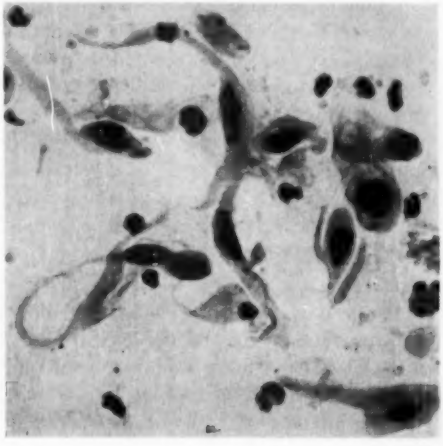


Fig. 5.—Elongate and bizarre cells. The nuclei are somewhat elongated and have an altered chromatin pattern. Pyknosis and vacuolization is evident in some cells. Invasive cancer; × 500.



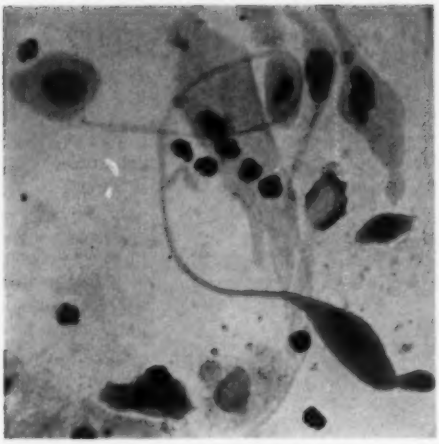


Fig. 6.—Unusually large caudate form and isolated primitive cells. The latter have abnormally large hyperchromatic nuclei with an altered chromatin pattern. Invasive cancer; × 500.

as were 96.56% of the cells encountered in dysplasia. In the presence of early epithelial infiltration, 86.40% of the cells were isolated, as compared with 87.20% of the cells observed in mice with cancer.

The cytoplasmic staining reaction was basophilic in 87.84% of the normal cells and acidophilic in only 12.16%. Of the cells observed in dysplasia, 75.76% were basophilic, 24.16% were acidophilic, and 0.08% were indeterminate in their staining reaction. In early infiltration, 63.76% of the cells were basophilic, 36.16% were acidophilic, and 0.08% were indeterminate. With outspoken cancer, only 56.96% of the cells were basophilic and 43.04% had an acidophilic cytoplasm. Vacuolation of the cytoplasm was observed in only 17.44% of the normal cells studied. Of the cells derived from a dysplastic change, 21.60% were vacuolated. With early infiltration, 92.00% of the cells had cytoplasmic vacuoles, as did 80.88% of the cells desquamated from cancer (Figs. 3 and 5).

The mean nuclear area as determined by planimetry is recorded in Table 1. In normal preparations the mean nuclear area was 40.64±8.50 square microns, while in dysplasia it was 92.15±13.66 square microns. In animals with early infiltration the desquamated cells had a mean nuclear area of 67.87±18.48 square microns, and in animals with cancer the mean was 60.35± 9.87 square microns.

The nuclear area can also be expressed in relation to the area of the cell, i. e., $\frac{\text{nuclear area}}{\text{cellular area}}$. In normal cells the relative nuclear area was 11.9%±2.42% of the cellular area. In cells derived from dysplasia the mean area occupied by the nucleus was 28.9%±5.85% of the cellular area. With early infiltration the relative nuclear area was 23.2%±1.86%, and in outspoken cancer 21.9%±2.92% of the cellular area was occupied by the nucleus.

Of the normal cells, 56.08% had round, isodiametric, nuclei, while 43.92% were nonisodiametric, including oval, irregular, or elongate forms. In dysplasia, 48.40% were round, or isodiametric, while 51.60% were nonisodiametric. In early infiltration, only 23.84% of the cells had isodiametric nuclei, while 76.16% were nonisodiametric. Of the latter, 41.52% were oval, 21.84% were irregular, and 12.80% were elongate. In cells from cancer only 18.72% of the nuclei were isodiametric. A total of 81.28% was nonisodiametric. Of these, 40.48% were oval, 22.16% were irregular, and 18.64% were elongate.

Although most of the cells had a single nucleus, 0.16% of the normal cells were multinucleated, as were 1.44% of the cells shed in the presence of dysplasia. With early infiltration, 3.36% of the cells were observed to be multinucleated, as were 4.80% of the cells derived from cancer.

In studying the arrangement of the chromatinic material in the nuclei, it was observed that a finely granular chromatin pattern was observed in 72.32% of the normal cells, while 27.68% had a coarsely granular pattern. In dysplasia, 66.80% of the cells had a finely granular chromatin pattern, while 33.20% had a coarsely granular pattern. A finely granular nuclear pattern was observed in 22.40% of the cells from early infiltrative lesions, and 77.60% of the nuclei were either coarsely granular,

translucent, or opaque. In frank cancer, 15.60% of the cells had a finely granular chromatin pattern and 84.40% had a nuclear mass which was either coarsely granular, translucent, or opaque.

Comment

The altered cells desquamated from dysplasia were predominantly isodiametric and were characterized by a relatively large hyperchromatic nucleus. The nuclear area was equal to 28.9%±5.85% of the cell area, while in the normal the mean was only 11.9%±2.42% of the cell area. This is a significant criterion by which cells arising in dysplastic lesions can be distinguished from those observed in normal specimens, the *P*-value being >0.0001. While there were other differences between the normal and the dysplastic epithelium, the magnitude of the change was somewhat less significant (Figs. 1 and 2).

The cells demonstrated in the presence of early invasive cancer were significantly different from the normal and were unlike those seen in the presence of a dysplastic change in that coarsely granular, translucent, or opaque nuclear forms were more abundant and nonisodiametric nuclear and cellular forms were more numerous. A significant increase in the number of elongate, caudate, and irregular cells was observed in early infiltration as compared to dysplasia. Also notable is a lower relative nuclear area in early infiltration (23.2%± 1.86%). In comparison with the mean in dysplasia, the *P*-value is >0.001.

The cellular changes observed in outspoken cancer are in marked contrast to those observed in either the normal or the dysplastic stage. As compared to early infiltration there are certain quantitative differences which are of such magnitude as to be significant. While the over-all distribution of nonisodiametric forms is comparable in the two categories, there is a significant increase in the number of elongate and caudate forms in the presence of advanced cancer. The relative nuclear area of the cells desquamated in advanced cancer is 21.9%±2.92% as compared to 23.2%± 1.86% in early cancer. The latter is not a significant difference.

The desquamated cells observed in the presence of frank cancer of the uterine cervix in the present study are basically similar to those which arise from cancer of the uterine cervix induced by intravaginal painting with 3,4-benzpyrene in acetone.[7,8]

Thus, the cellular elements desquamated into the vagina of the normal mouse are significantly different from those encountered during carcinogenesis. Similarly, the cells originating in dysplasia are different from those noted in either early infiltration or frank cancer, and there are also certain quantitative differences between the cells of an early infiltrative process and those from induced cancer.

As cancer develops, there are certain fundamental changes which occur in the desquamated cells. As indicated by von Haam and Scarpelli, there is a progressive increase in the number of altered cells. This is associated with a gradual decrease in cell size and a greater uniformity of cell area. The desquamation of progressively smaller cells in all probability is a reflection of the increased content of more primitive cells in the altered mucosa, while the more uniform cell size is in part due to the loss of cyclic activity in the uterine mucosa.

During carcinogenesis in the mouse cervix there is a decrease in the proportion of isodiametric forms, while nonisodiametric configurations involving both the nucleus and the cytosome become more abundant. The unusual cell forms are due to the fact that most of the resulting cancers are well-differentiated squamous-cell cancers and many of the desquamated cells reflect an abnormal keratinization in the epithelium. This is commonly manifested by the shedding of elongate or caudate cells, whose unusual shape may be explained by the presence of a delicate linear cytoplasmic fibril.[4] This is a remnant of the fibrillar

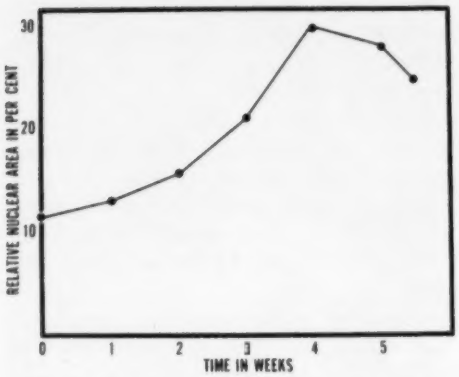


Fig. 7.—Changes in relative nuclear area in carcinogenesis.

apparatus which is currently believed to be concerned with the production of keratin.

Early during carcinogenesis there is a marked increase in the nuclear size, followed by some reduction in nuclear area. This is well illustrated in Figure 7. The relative nuclear area was computed on seven consecutive samplings in one animal (D24) and plotted in relation to time. The animal was killed five and one-half weeks after the insertion of a carcinogen-impregnated thread and was demonstrated to have cancer. The relative nuclear area reached a maximum of 29.8% at the end of four weeks and then dropped. This is of some interest because the decrease in the nuclear area is chronologically related to early infiltration. A degenerative change leading to nuclear pyknosis, which occurs at about this time, may explain the reduction in nuclear mass, although other factors may also be of importance.

As cancer develops, isodiametric nuclear forms are relatively less common and non-isodiametric shapes more numerous. This is in part explained by changes in cell form. A finely granular chromatin pattern, which is characteristic of the interphasic nucleus, is common in the normal but is relatively less common as cancer develops. An increasing number of the cells have hyperchromatic coarsely granular nuclear masses in the latter stages of carcinogenesis. These were often associated with prominent chromocenters or nucleoli. Similarly, there was a greater proportion of translucent or opaque nuclear masses indicating stages of pyknosis.

Summary

The cells desquamated into the vagina of the mouse during the induction of cancer in the cervix uteri are significantly different from those encountered during normal estrus. The basic cellular changes are considered in relation to the over-all process of carcinogenesis in this site, and a detailed analysis is made of the cellular alterations in each of three arbitrary stages of carcinogenesis.

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Histochemistry of Breast Diseases

I. Phosphatases

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Introduction

Acid and alkaline phosphomonoesterases * are found in many organs of the human body in normal and pathologic states. This paper presents our observations of the distribution of phosphatases in diseases of the breast.

Breast cancer usually contains acid phosphatase but seldom alkaline phosphatase activity.[1-4] Drennan found alkaline phosphatase activity in only 4 of 100 cases of breast cancer,[5] and Glück demonstrated the acid enzyme in 27 and the alkaline in only 4 of 33 biopsy specimens of breast cancer.[6] These authors suggested that the variation in alkaline-phosphatase activity in cancer of the breast might indicate tumors amenable to hormone therapy.

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* For brevity, the term "phosphomonoesterase" is shortened to "phosphatase."

Materials and Methods

The breast tissue used in this study was primarily obtained from surgical biopsies. Care was taken to receive the tissue within a half hour of removal in order to insure an optimal histochemical reaction. Relatively normal tissue was obtained from radical mastectomy cases at a distance from the tumor. Some tissues from autopsy were used, providing the case was less than six hours post mortem. Sections of kidney obtained at time of surgery were used for positive controls. Miscellaneous tumors were similarly obtained.

The tissues were carefully cut into small blocks no larger than 15×5×5 mm. Pieces fixed immediately in Zenker's fluid were embedded in paraffin. Sections made from these were stained with hematoxylin and eosin and studied for breast morphology and for diagnosis of the case. The tissues to be studied for the presence of enzymes were stored in glass vials and kept in solid carbon dioxide (Dry Ice) at a temperature of approximately —70 C. After storage for one week, the

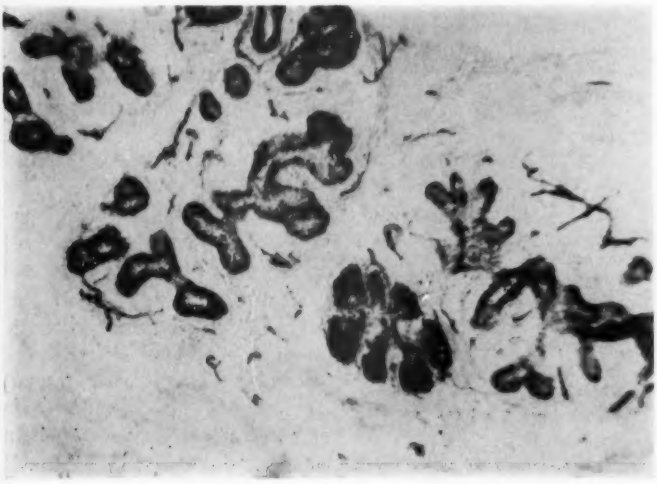

Fig. 1.—Normal breast. Alkaline phosphatase-activity in lobules after six months' storage in solid carbon dioxide. Diazo Blue B; reduced 10% from mag. × 65.

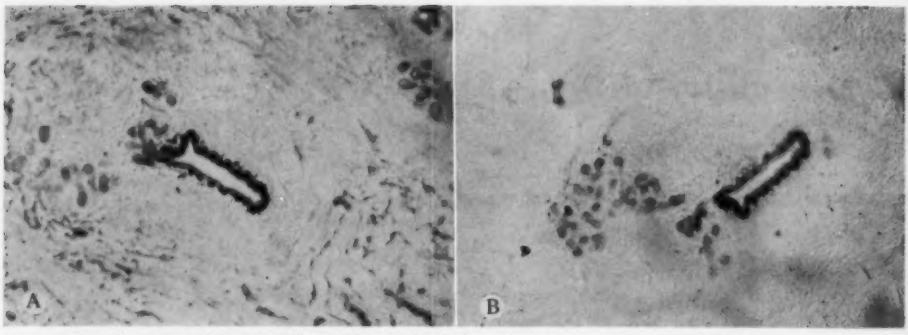

Fig. 2.—Normal breast. Comparison of alkaline-phosphatase reaction with two techniques. Note the serrated outline, indicating reaction in the myoepithelial region of a terminal duct. (*a*) Diazo Blue B, (*b*) calcium phosphate; reduced 40% from mag. × 65.

tissues were fixed in 10% neutral, unbuffered formalin at 4 C for two hours. Storage in solid carbon dioxide does not reduce enzymatic activity, and the results agreed favorably with duplicate sections prepared immediately after surgical removal. Phosphatase activity remained satisfactorily demonstrable for six months in solid carbon dioxide (Fig. 1).

The tissues were sectioned on the freezing microtome at 15μ-125μ. Sections 30μ-40μ thick gave the best results. A razor-blade holder was adapted to the microtome to assure a constantly sharp edge.

Acid Phosphatase.—Both the azo and lead methods of Gomori were used.[7] In the azo method, sodium α-naphthyl acid phosphate was used as the substrate and Diazo Blue B or Fast Blue RR was used as the dye. Sections were incubated in this mixture for 30 minutes. Diazo Blue B yielded purple sites of activity, while Fast Blue RR gave a black reaction. In the lead method, sections were incubated for two hours. An acetic acid rinse was used to reduce nuclear artifact. The sites of activity appeared brown or black. Both the azo and the lead techniques gave similar localization, but the lead method was found to be more reproducible.

Alkaline Phosphatase.—Both the azo and calcium phosphate methods of Gomori were used.[7] In the azo method, sodium α-naphthyl acid phosphate was used as the substrate and Diazo Blue B or Fast Blue RR was used as the dye. Sections were incubated for 30 minutes. To avoid excess precipitation the sections were put in fresh substrate twice during the incubation. With Diazo Blue B the sites of activity were purple to black, while Fast Blue RR produced a black reaction. The Diazo Blue B gave a sharper localization than the Fast Blue RR, which tended to give a diffuse precipitate. In the calcium phosphate method, sections were incubated for two hours. Sites of activity were black. Both the azo and calcium phosphate methods gave similar localization, but the azo method with use of Diazo Blue B was more distinct (Fig. 2).

Sections of kidney were used as positive controls for both acid and alkaline phosphatase. Two negative controls were used: a reagent control in which the substrate was omitted and an inhibition control in which kidney sections were placed in boiling water for two minutes and then treated with the standard procedures. Controls were periodically used throughout the study.

The stained sections were stored in water or 10% formalin overnight to allow the gas bubbles to escape. They were then washed in distilled water, floated onto slides, and mounted in glycerin jelly.

Observations

Normal Breast.—Histology of Normal Breast: Huseby and Thomas have given a succinct description of breast histology in the young woman and of the changes with age.[8] The main ducts from the nipple divide many times in the breast and terminate in clusters of small blind pouches, the alveolar ducts. We have classified the duct leading into a lobule as the terminal duct. Each cluster of alveolar ducts with its connective tissue stroma forms a breast lobule (Fig. 3). The connective tissue in the lobule is loose and fibrillar in contrast with the dense collagenous stroma of the interlobular zones. The ducts have a similar morphology throughout their ramifications. They are lined with two layers of cells: an inner, or luminal epithelial layer, and a

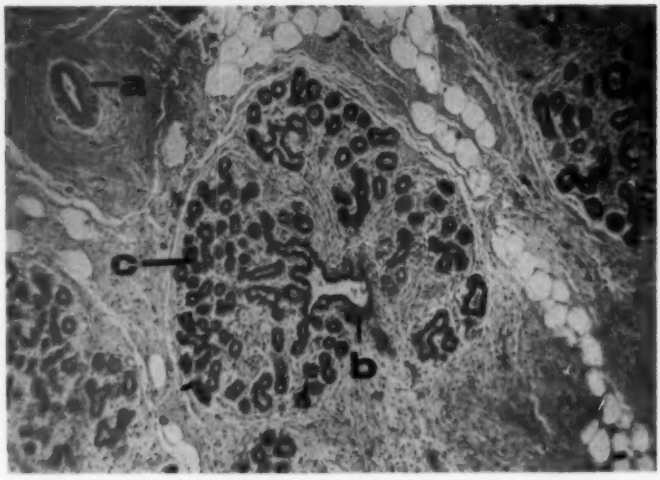

Fig. 3.—Normal breast. Morphology of a breast lobule: (*a*) interlobular duct, (*b*) terminal duct, (*c*) alveolar ducts. H & E; reduced 10% from mag. × 65.

basal, or myoepithelial layer (Fig. 4). The inner layer is cuboidal or columnar, and the basal layer is cuboidal or flat. With the hematoxylin and eosin stain the myoepithelial cells are seen to consist of small nuclei, which are oval or flat, and a small amount of poorly defined cytoplasm. The alveolar ducts consist of small gland-like structures that are lined with cuboidal epithelial cells. A few myoepithelial cells surround the alveolar structures, and they are, therefore, classified as ducts. The myoepithelial cells are most numerous and easily recognizable in the larger ducts. They are fewer in number in the small duct radicles. With increasing age there is progressive condensation of the intralobular connective tissue, shrinkage of the alveolar ducts, and disappearance of the myoepithelial cells in the lobules. In women 50 years or older the ducts just proximal to the lobules are occasionally dilated. This was found in autopsy specimens from women clinically free of fibrocystic disease.

Histochemistry of Normal Breast: Appreciable amounts of acid phosphatase were found in the normal breast in all age groups, both in the nuclei and in the cytoplasm of

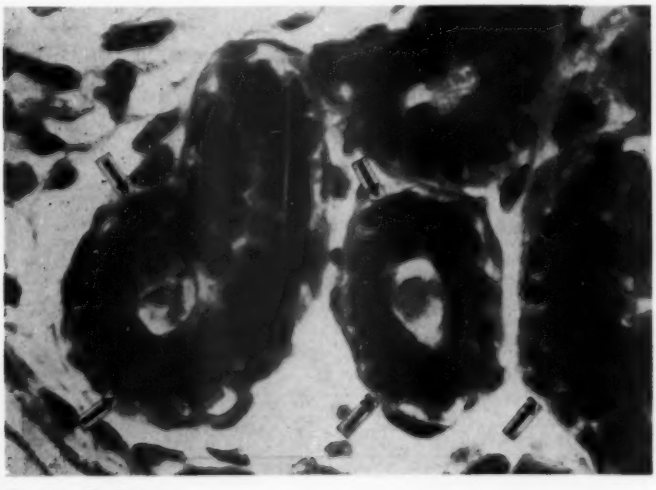

Fig. 4.—Normal breast. Myoepithelial cells in alveolar ducts. H & E; reduced 10% from mag. × 550.

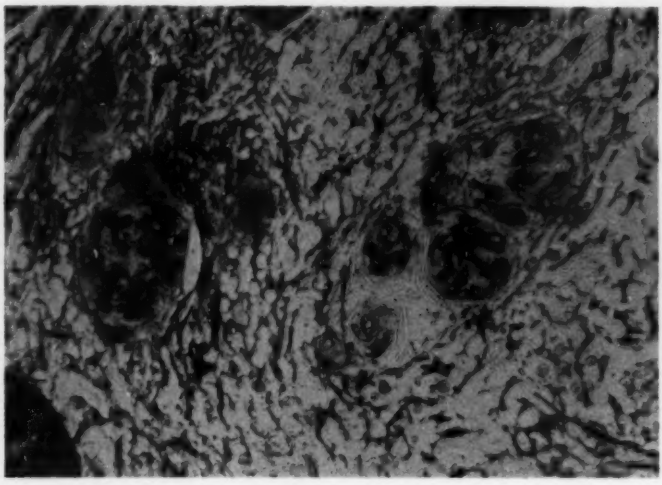

Fig. 5.—Normal breast. Alkaline phosphatase reaction in collagen fibrils as well as in a normal breast lobule. Fast Blue RR; reduced 10% from mag. × 8.5.

all epithelial elements. Thus, activity was seen in interlobular, terminal, and alveolar duct epithelium. There was no significant variation in reaction. Fibroblasts, particularly the large immature form, often showed a nuclear reaction. Normal breast tissue contained variable amounts of alkaline phosphatase that cannot be localized with precision. Similar structures in the same section, and even in the same microscopic field, showed variable amounts of the enzyme. Both the azo and the calcium phosphate methods showed a diffuse reaction, making the exact localization difficult. The reaction was most intense and best localized in the interlobular and terminal ducts, whose wrinkled epithelium consists of an inner layer of columnar cells and a basal layer of myoepithelial cells. The reaction was most intense in the myoepithelial cells and extended irregularly into the epithelial cells, producing a serrated outline (Fig. 2). All three techniques for alkaline phosphatase gave the same type of reaction. Alveolar ducts often showed a more diffuse activity in the epithelial cytoplasm. A basal reaction was rare in these ducts, probably because myoepithelial cells are not numerous. Fibroblastic nuclei and collagen fibrils were occasionally reactive (Fig. 5). The endothelium of capillaries contained alkaline phosphatase and stained well. Studies of aging breasts revealed a diminution of alkaline phosphatase in the involuted breast lobules but a persistence of activity in the interlobular and terminal ducts. Aging changes in the breast lobules were irregular. Occasionally, there were well-developed breast lobules with alkaline-phosphatase activity.

Fibrocystic Disease.—There were 88 cases of fibrocystic disease, including 4 cases of adenosis (Table 1). Myoepithelial cells were noted in the lining of small and medium-sized cysts and rarely in the large cysts in fibrocystic disease.

Acid Phosphatase: Acid phosphatase activity was constantly strong in adenosis (Fig. 6). In other types of fibrocystic disease, there was a variable reaction in the lobules, ducts, and cysts. Although no attempt was made to quantitate it, the activity

TABLE 1.—*Occurrence of Acid- and Alkaline-Phosphatase in Diseases of the Breast*

Diagnosis	Cases, No.	Acid P-Ase.		Alk. P-Ase.	
		Pos.	Neg.	Pos.	Neg.
Fibrocystic disease	84	84	0	Variable	
Adenosis	4	4	0	4	0
Gynecomastia	5	5	0	5	0
Intraductal papilloma	3	3	0	3	0
Fibroadenoma	23	23	0	Variable	
Carcinoma	44	44	0	10	34

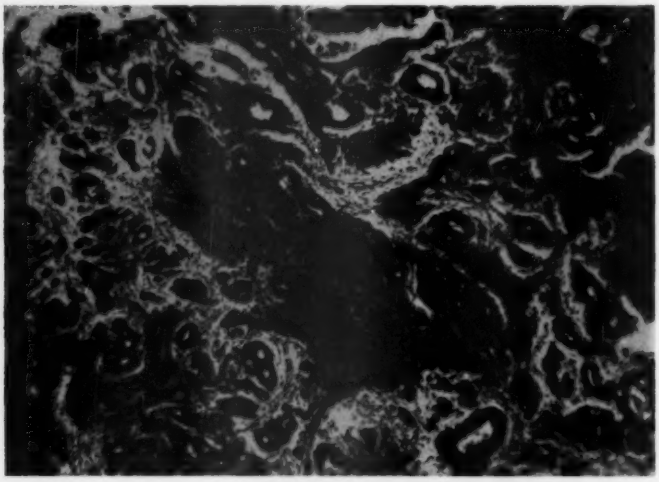

Fig. 6.—Adenosis. Acid phosphatase reaction in areas of adenosis and in ductal structures. Lead method; reduced 10% from mag. × 65.

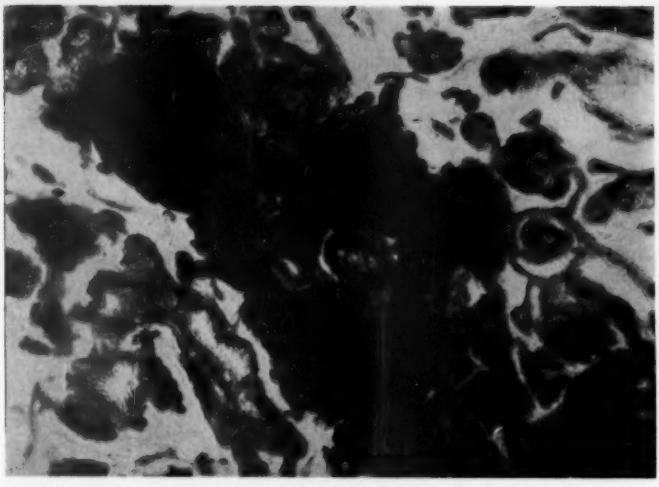

Fig. 7.—Adenosis. Alkaline phosphatase reaction. Calcium phosphate; reduced 10% from mag. × 140.

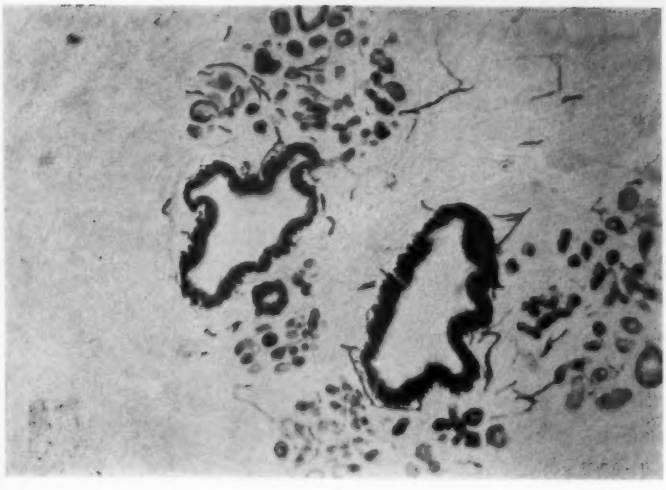

Fig. 8.—Fibrocystic disease. Alkaline-phosphatase activity in the myoepithelial region and adjacent epithelium of small cysts. Alveolar ducts show variable activity. Note the reaction in capillaries. Diazo Blue B; reduced 10% from mag. × 65.

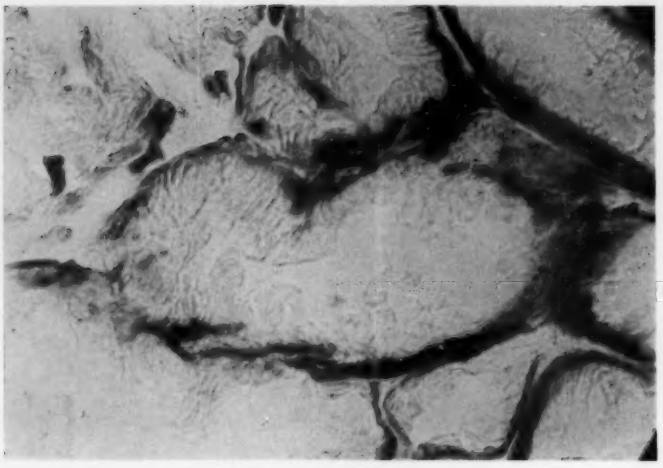

Fig. 9.—Fibrocystic disease. No alkaline-phosphatase activity in apocrine cells; activity in myoepithelium. Diazo Blue B; reduced 10% from mag. × 286.

tended to be more intense in the areas of intraductal hyperplasia.

Alkaline Phosphatase: In all cases of adenosis, alkaline-phosphatase activity was so intense that it obscured cellular detail (Fig. 7). The activity was inconstant in cysts, and the localization was poorly defined. It occurred in the periphery of the cyst wall, involving the basal portion of the lining epithelium and the adjacent myoepithelium (Fig. 8). In the dilated cysts, the myoepithelial cells were usually scanty and difficult to recognize. The very large cysts showed the least amount of activity. There was variable alkaline-phosphatase activity in intraductal hyperplasia. Activity was not demonstrated in the apocrine cells of cysts and ducts but was present in the myoepithelium (Fig. 9). Young fibroblasts of the stroma were frequently reactive. There was also a patchy staining of the collagen fibrils. Capillaries in all cases showed alkaline-phosphatase activity.

Gynecomastia.—There were five histologically characteristic cases of gynecomastia. In the hyperplastic ductal epithelium, the myoepithelial cells were intermingled

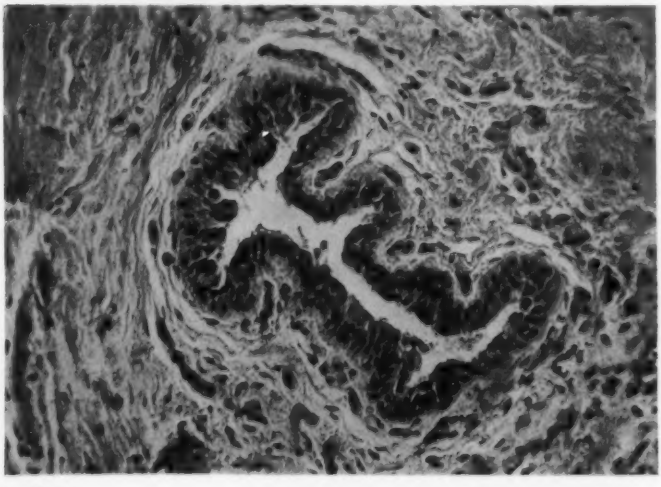

Fig. 10.—Gynecomastia. Cellular hyperplasia of duct; it is difficult to distinguish myoepithelial cells from epithelial cells. H & E; reduced 10% from mag. × 65.

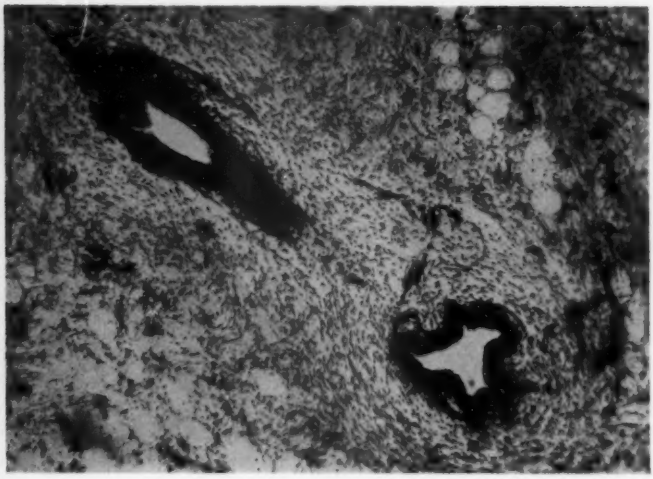

Fig. 11.—Gynecomastia. Acid-phosphatase reaction in ducts and in stromal cells. Lead method; reduced 10% from mag. × 65.

with epithelial cells, making their delineation difficult (Fig. 10).

Phosphatase.—Both the acid- and alkaline-phosphatase reactions were consistently positive. Acid phosphatase was demonstrated in the epithelium of ducts and occasionally in fibroblasts of the adjacent stroma (Fig. 11). A strong alkaline-phosphatase reaction at the periphery of the ducts corresponds to the myoepithelium (Fig. 12).

Intraductal Papilloma.—The three histologically characteristic cases of intraductal papilloma showed epithelial cells reactive for both acid and alkaline phosphatase.

Fibroadenoma.—Of the 23 fibroadenomas, 11 were intracanalicular; 7, pericanalicular; 4, of mixed type, and 1, lactating adenoma. These conformed to the usual microscopic characteristics of these tumors. Both intra- and pericanalicular types showed similar enzymatic activity. Myoepithelial cells were present in variable numbers in all cases (Fig. 13).

Acid Phosphatase: Acid phosphatase was consistently demonstrated in the lining epithelium of the compressed ducts and occasionally in nearby fibroblasts (Fig. 14).

Fig. 12.—Gynecomastia. Alkaline-phosphatase reaction in myoepithelial region of duct. The epithelium has been folded in the upper right margin. Diazo Blue B; reduced 10% from mag. × 140.

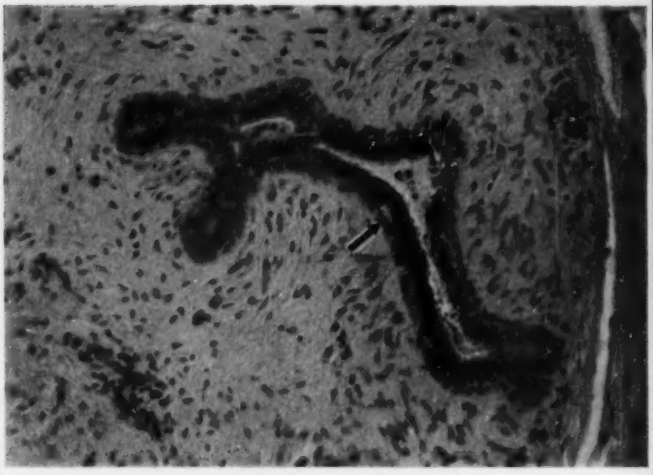

Fig. 13.—Fibroadenoma. Myoepithelial cells in a compressed duct. H & E; reduced 10% from mag. × 200.

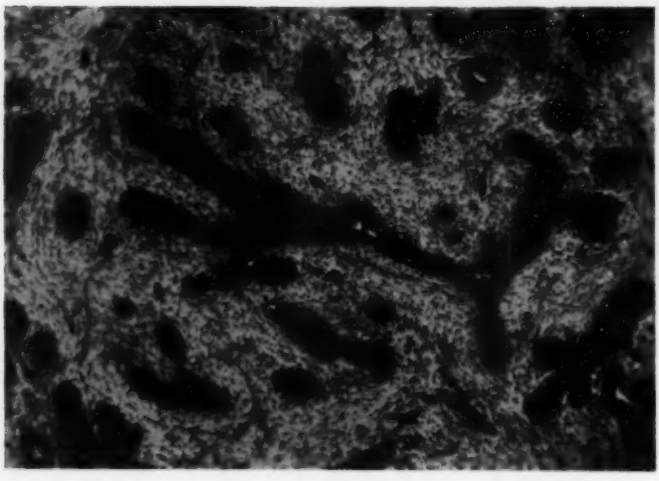

Fig. 14. — Fibroadenoma. Acid-phosphatase reaction in ducts and in stromal cells. Lead method; reduced 10% from mag. × 140.

There was a fairly intense activity in the epithelial cells of the lactating adenoma.

Alkaline Phosphatase: Alkaline-phosphatase reaction was usually weak and diffuse in the ductal epithelium, although strong activity was demonstrated in the ducts of the fibroadenomas, which had clearly demarcated myoepithelial cells. The normal breast lobules were usually reactive. Occasionally there was slight cystic dilatation of the ducts, with inconstant activity. There was often a slight activity in the stromal cells immediately surrounding the ducts.

TABLE 2.—*Occurrence of Acid and Alkaline Phosphatase in Breast Cancer*

Diagnosis	Cases, No.	Acid P-Ase. Pos.	Acid P-Ase. Neg.	Alk. P-Ase. Pos.	Alk. P-Ase. Neg.
Intraductal carcinoma	5	5	0	1	4
Paget's disease	1	1	0	0	1
Infiltrating ductal carcinoma	21	21	0	6	15
Scirrhus adenocarcinoma	9	9	0	0	9
Adenocarcinoma	3	3	0	1	2
Poorly differentiated carcinoma	5	5	0	2	3

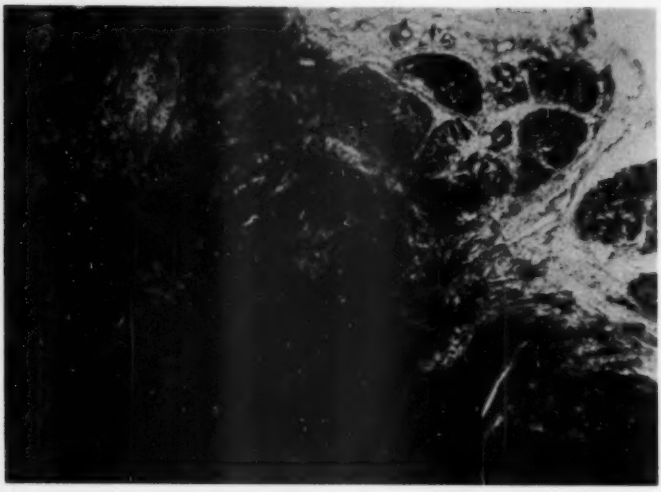

Fig. 15. — Carcinoma. Acid-phosphatase reaction in tumor cells and in adjacent breast lobules. Lead method; reduced 10% from mag. × 8.5.

Because of the slight branching of the ducts the lesion occasionally resembled rudimentary breast tissue. The phosphatase activity in this type did not differ from the other morphologic types. In the lactating adenoma there was activity in the epithelial cells.

Carcinoma.—There were 44 cases of breast cancer (Table 2). These were classified as intraductal carcinoma, 5; infiltrating ductal carcinoma, 21; scirrhous adenocarcinoma, 9; adenocarcinoma, 3; poorly differentiated carcinoma, 5, and Paget's disease, 1.

Acid Phosphatase: The reaction was consistently strong in all 44 cases and varied neither with the differentiation of the tumor nor with the age of the patient. The activity was most marked at the periphery of the tumor, where it was infiltrating as cords and single cells. The intense reaction in the tumor contrasted sharply with the moderate reaction in the neighboring nontumorous breast (Fig. 15). Young fibroblasts in the stroma around the infiltrating tumor were reactive. We have seen moderate variation in the acid-phosphatase activity in the carci-

Fig. 16.—Paget's disease. Intense acid-phosphatase activity in tumor cells within and beneath the epidermis. The superficial epidermis is also reactive. Lead method; reduced 10% from mag. × 65.

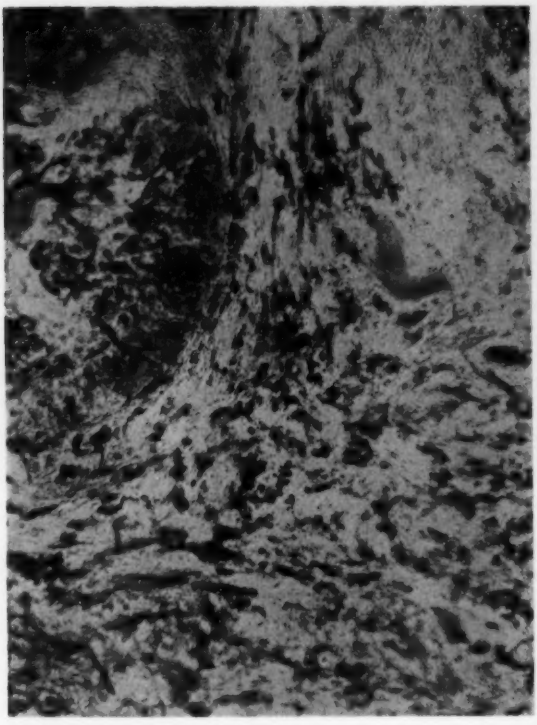

Fig. 17.—Carcinoma. Alkaline-phosphatase reaction in infiltrating tumor cells and in an adjacent lobule. Diazo Blue B; ×65.

nomas but not to the degree noted by Reiner et al.[9] In the case of Paget's disease the acid phosphatase reaction was intense in the tumor masses beneath the nipple and in the adjacent skin. Nests of tumor cells in the epidermis were reactive (Fig. 16). Vacuolated Paget's cells were not recognized, possibly being obscured by the reaction.

Alkaline Phosphatase: An infrequent positive reaction was seen in carcinoma of the breast. Of 44 cases, 10 showed moderate but patchy activity, chiefly in the periphery of the tumor (Fig. 17). Alkaline-phosphatase activity was demonstrated most consistently in the breast cancers of older women. The case of Paget's disease revealed no alkaline-phosphatase activity in the tumor in the epidermis and subcutaneous tissue. In cases of carcinoma the tissue surrounding the tumor contained capillaries of normal configuration. Within the tumor, the capillaries were less frequent and appeared as distorted segments. In older women, lobules of uninvolved breast parenchyma near the cancer showed more alkaline-phosphatase activity than normal breast tissue in women of the same age.

Comment

In the sex-related organs, acid and alkaline phosphatase appear to be dependent on steroid hormones.[10,11] Acid-phosphatase activity in the adrenal cortex of the male mouse is dependent on androgen.[10] There is a cyclic variation in alkaline phosphatase in the endometrium of women[12]; in the monkey, the enzyme disappears in the endometrium after castration and reappears following the administration of estrogen.[12]

Alkaline phosphatase is said to decrease progressively in the breast with aging.[8] Concurrently there is a progressive atrophy and shrinkage of breast lobules and a decrease in prominence of the myoepithelial cells. Alkaline phosphatase in breast lobules is chiefly found in these cells. When estro-

gen is administered to postmenopausal women, there occurs a stimulation of breast lobule formation, myoepithelial proliferation, and reappearance of alkaline phosphatase in the lobules.[8] It appears reasonable to consider alkaline-phosphatase activity in the breast to be under the influence of the female sex hormone. It is not known whether estrogen acts directly on the breast epithelium or is mediated through the pituitary or adrenal glands.

The myoepithelium is the major site of alkaline-phosphatase activity in fibrocystic disease, gynecomastia, and fibroadenomas. In adenosis, alkaline-phosphatase activity is present in ductal epithelium as well as in the surrounding cellular aggregates. The intense alkaline-phosphatase activity in these masses of cells suggests their possible myoepithelial nature.

Fibrocystic disease, which is considered to be due to an imbalance in the sex hormones, shows a variable distribution of phosphatase activity. Neither acid- nor alkaline-phosphatase activity in normal lobules or in cysts in fibrocystic disease could be correlated with the age of the patient.

Gynecomastia is characterized by a hyperplasia of the myoepithelium of ducts.[13] With the hematoxylin and eosin stain, the myoepithelial cells were seen intermingled with epithelial cells in the hyperplastic lining of ducts. The alkaline-phosphatase reaction, however, was always present in the peripheral zone of the ducts, the region normally occupied by myoepithelial cells. The lack of correlation of the location of alkaline-phosphatase activity with the myoepithelial-cell hyperplasia is not understood. The prominent alkaline phosphatase in gynecomastia gives additional support to the concept that this condition may be produced by hyperestrinism.[14]

In adenosis and intraductal papillomata both acid and alkaline phosphatase were demonstrable in all cases examined. A larger series of cases must be studied to determine the constancy of these reactions.

All of the fibroadenoma cases were from patients in the premenopausal group. Acid-phosphatase activity was present in the ductal epithelium of all the cases. The alkaline-phosphatase reaction varied from duct to duct. It was most prominent in ducts which contained frequent myoepithelial cells.

In breast cancer, there is a striking contrast between the constant presence of acid phosphatase and the infrequent presence of alkaline-phosphatase activity. These findings in tumors of the breast are in agreement with those of other investigators.[1-6,9,15,16] However, the occurrence of acid phosphatase and the relative rarity of alkaline phosphatase in breast cancer are not unique to this neoplasm. Acid phosphatase occurs in carcinoma of the prostate, breast, kidney, ovary, stomach, and colon.[9] The largest amount of the enzyme occurs in prostatic tumor, both benign and malignant. We have demonstrated the presence of acid phosphatase and absence of alkaline phosphatase in adenocarcinoma of the stomach, pseudomucinous cystadenocarcinoma of the ovary, metastatic undifferentiated adenocarcinoma of the lung, fibrosarcoma of the breast, and leiomyosarcoma of the uterus. Epithelial neoplasms are noteworthy because of the absence of alkaline phosphatase in them.[15]

Although a number of epithelial neoplasms have a pattern of acid- and alkaline-phosphatase activity similar to breast cancer, it may have a special significance in this sex-linked organ. In the normal breast, alkaline-phosphatase activity is under the influence of estrogen, whereas acid phosphatase is not. Thus, acid-phosphatase activity in the breast does not vary with age. If alkaline phosphatase in breast cancer were dependent on ovarian estrogen production, the tumors showing this activity should be in young women. Eighty per cent of our carcinoma cases showing this enzyme were in women over 50 years old. This observation tends to militate against a relationship between alkaline-phosphatase activity and estrogen in breast cancer. Specimens came from equal groups of pre- and postmenopausal patients. The concurrent study of vaginal cytology and alkaline-phosphatase

activity in cases of breast cancer in postmenopausal women might furnish valuable information concerning a relationship between this enzyme and estrogen.

Drennan was of the opinion that breast cancers which contained alkaline-phosphatase activity might respond favorably to sex-hormone therapy.[5] We are following our cases of breast cancer in order to determine the validity of this impression.

The histochemical demonstration of strong acid-phosphatase activity in breast cancer and in other epithelial neoplasms helps to explain the occasional occurrence of elevated serum acid phosphatase in patients with widespread cancers other than of the prostate.[16]

Phosphatase activity is not limited to epithelial cells. Several authors, including ourselves, have demonstrated the presence of both acid and alkaline phosphatase in fibroblasts.[4,6,9,17,18] We have noted alkaline-phosphatase enzyme in collagen fibrils. Gross has demonstrated that young collagen fibrils absorb this enzyme from the blood.[18] The presence of the enzyme in this anuclear tissue had formerly been ascribed to artifact.

Alkaline-phosphatase activity in capillary endothelium of the breast delineates clearly the course of these vessels (Fig. 18). The distribution of capillaries in breast disease is to be described in a subsequent article.

Summary

Histochemical reactions for acid and alkaline phosphatase have been studied in normal breast, fibrocystic disease, intraductal papillomas, fibroadenomas, gynecomastia, and breast cancer.

Acid phosphatase was demonstrated in all cases, with intense activity in cancer. Alkaline-phosphatase activity was variable. The reaction was most constant in the myoepithelium of fibrocystic disease, fibroadeno-

Fig. 18.—Fibrocystic disease. Capillaries in a cyst wall. Alkaline phosphatase, Fast Blue RR; ×65.

mas, gynecomastia, and normal breast of premenopausal women.

Alkaline-phosphatase activity and myoepithelium in the breast were correlated with female sex-hormone activity.

Fibrocystic disease and fibroadenomas had variable amounts of alkaline-phosphatase activity. The variation may be a reflection of sex-hormone imbalance.

A small series of cases of adenosis and intraductal papillomas have shown positive acid- and alkaline-phosphatase activity. Further studies shall be made to determine the constancy of these reactions.

In gynecomastia, persistent alkaline-phosphatase activity was demonstrated in the myoepithelial cells, supporting the theory that this lesion is due to hyperestrinism.

In breast cancer, acid-phosphatase activity was consistently present, whereas alkaline-phosphatase activity was infrequent. The relationship between alkaline-phosphatase activity in breast cancer and estrogens was discussed. The contrasting occurrence of acid- and alkaline-phosphatase activity in breast cancer is not unique to this neoplasm. Many epithelial malignant tumors have acid-phosphatase activity and rare alkaline-phosphatase activity.

Additional studies correlating clinical and histochemical observations in breast cancer will be reported.

Mr. Arthur F. Jacques prepared the photomicrographs.

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Experimental Cyanide Encephalopathy

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Many chemical and physical agents have been used to cause demyelinating lesions in the brains of experimental animals. Probably the closest approximation to spontaneous demyelinating disease is provided by cyanide intoxication.[1] Previous methods of administering potassium cyanide have required protracted series of injections, and lesions have been uncertain in occurrence and unpredictable in distribution. In the work reported here it has been demonstrated that a single brief exposure to hydrogen cyanide vapor will produce lesions of gray and/or white matter regularly in rats and that by control of the depth and duration of the intoxication it is possible to control the distribution of the lesions to a limited extent.

Review of Literature

Brain lesions have been produced by injections of potassium cyanide in the monkey,[2,3] cat,[2,4] dog,[5-7] rabbit,[5,8] and rat.[9-11] Potassium cyanide was usually given in progressively increasing doses over long periods of time, although Hurst[3] reported that some lesions in monkeys were produced by single doses. The lesions involved central white matter, corpus callosum, corpus striatum, optic pathways, anterior commissure, cerebral cortex, cerebellum, hippocampus, brain stem, and cord. They varied in severity from partial or complete demyelination and neuronal necrobiosis to complete necrosis of white or gray matter. Ferraro[2] noted sparing of the U-fibers and stated, ". . . no clear differentiation is possible between the lesions described in human diffuse sclerosis and the ones reproduced experimentally with potassium cyanide." Hurst[3] pointed out a similarity between cyanide lesions and acute epizootic leukoencephalitis of horses. Jedlowski[6] described stripe-like degeneration and concentric plaques resembling Balo's disease. Lumsden[9] produced lesions of the corpus callosum in rats, which he compared to Marchiafava-Bignami disease.

Lumsden,[9] using progressively increasing doses, found one lesion in 100 rats studied during the first 2 weeks of the experiment, while there were lesions in 16 out of 37 rats who survived 2 to 26 weeks. All the lesions were in the corpus callosum and hippocampal commissure. However, Rose, Harris, and Chen,[10] using a similar procedure, obtained brain lesions in only 5 of 52 rats who survived from 1 to 17 weeks, and of these only 3 had demyelinating lesions of the callosum. These workers started with small rats (100 gm.), which, as will be shown, may have been responsible for the low yield.

Hicks[11] also treated rats with sodium cyanide injections but by a very different technique. Multiple injections were given over a period of hours or of successive days. As soon as the rat showed signs of recovery it was given another injection, so as to be maintained in a fairly continuous state of coma or convulsions. Of 53 rats there were 17 acute deaths, while 14 survived the procedure without developing lesions and 22 survived with lesions of the callosum (5 rats), striatum, cortex, hippocampus, substantia nigra, optic nerve, cerebellum, or retina.

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Hicks considered that successful production of cerebral lesions required that the rat be kept in a continuous state of deep cyanide intoxication for several hours. This fact suggested to us the advisability of a continuous rather than intermittent technique of administration so as to maintain a more constant intoxication, and this led to our use of the respiratory route.

Experimental Procedures

The respiratory route was used for the administration of cyanide because it permits continuous administration and accurate control of the depth and duration of the intoxication. Female albino rats from four different commercial sources were used. Only a few male rats were used.

Comparison of Inhalation and Injection Methods.—When potassium cyanide is injected, the blood and tissue level of cyanide will depend on the balance between absorption and detoxification. It will rise at first, owing to rapid absorption from the injection site, reach a maximum, and fall off as the rate of absorption is exceeded by the rate of detoxification. If the peak level is beyond a critical point, irreversible damage to vital centers occurs and is lethal. If the peak level is below this critical point, that is, sublethal, the vital centers may be spared, but this sublethal level of cyanide may not be maintained for a sufficiently long time to induce lesions in the corpus callosum and other susceptible areas of the brain. When hydrogen cyanide is administered by inhalation and controlled by constant observation of the response of the animal, the blood and tissue level will be determined by the controlled rate of administration, regardless of the rate of detoxification. Therefore, the severity of intoxication can be controlled on a moment-by-moment basis and sublethal concentrations can be achieved regularly and maintained fairly constant for any length of time.

Exposure Chambers.—Hydrogen cyanide was generated by bubbling air through a 5% aqueous solution of potassium cyanide. The hydrogen cyanide vapor was mixed with pure air in varying proportions, depending on the response of the test animals.

Initial attempts were made with a 20 liter battery jar in which 6 to 10 rats were exposed simultaneously. This proved unsatisfactory because of individual variation in susceptibility to hydrogen cyanide. Frequently some of the rats died under conditions which had little or no observable effect on others.

Much better results were obtained by placing each rat in a small separate glass jar (1 qt. or 1 gal. wide-mouth jar closed by No. 14 rubber stopper). A separate fritted-glass bubbler for each rat served to generate hydrogen cyanide vapor. The intoxication was controlled, as above, by mixing the cyanide vapor with pure air, but the mixtures were determined on a moment-by-moment basis for each rat individually. A method of arranging six such units to operate simultaneously on a single source of air has been described.[18]

Observations During Exposure.—When the concentration of hydrogen cyanide was increased slowly, the exposed rat went through a succession of four stages, each with characteristic signs. In Stage 1, the rat responded to the vapor by restlessness and increasing activity, leading to violent attempts to escape from the chamber. In Stage 2, voluntary muscular activity and postural tonus were greatly decreased, so the rat sank to the floor of the chamber. However, the animal responded with movement to tilting or tapping the jar. Ineffectual running movements and convulsive movements of extremities and tail occurred. Respiration varied from very deep, slow, and regular to irregular with short period of apnea.

In Stage 3, the loss of motor power was more complete, so there was no response to tilting the jar or causing the animal to roll over. Slight movements or twitches of extremities and tail persisted for a while in this stage. A brisk tap on the jar elicited a twitch of the ear; the twitch became progressively weaker. Respiration was regular and of moderate depth and varied from 40-80 per minute in frequency.

In Stage 4, there were no movements or twitches, and the ear did not respond to tapping the jar. Respiration was slow and regular and diminished progressively in amplitude and frequently till death supervened.

If the cyanide was discontinued before irreversible damage was inflicted on vital centers, the stages were retraced but in much less regular fashion. The first sign of emergence from Stage 4 was a return of the ear reflex, movement of extremities or tail, or hyperpnea.

Deaths During Exposure.—Death may occur from respiratory paralysis in Stage 4. If the cyanide was administered till the respiration was shallow, death was almost certain. Sometimes death occurred even when the animal was removed from the chamber at the very first sign of weakening respiration. Apparently, there was a level of intoxication which, if attained only very briefly, caused irreversible damage to the respiratory center. In these cases, the heart continued to beat for a minute or

more, but artificial respiration has been of no avail.

Deaths not related to cyanide overdosage occurred when the rat returned to Stage 2 during the exposure or after termination. In some of these cases, there were convulsive movements, with periods of apnea followed by complete flaccidity, very shallow respiration, and early death. It is probable that the apnea produced an added burden of anoxic anoxia which destroyed the respiratory center. Other examples of Stage 2 deaths were characterized by sudden apparently simultaneous cessation of cardiac and respiratory activity. These appeared to be due to cardiac exhaustion and occasionally to cardiac rupture. They usually followed a light but long exposure (especially after two hours) characterized by frequent or incessant twitching and convulsive movements (Stage 2). Some deaths during or soon after exposure were due to cerebral hemorrhage.

Conduct of Exposure.—With a total air flow of at least 1 liter per minute per jar, hydrogen cyanide was administered at a rate sufficient for the rats to reach Stage 1 in about 5 minutes and Stage 2 in about 10 minutes after onset. At this point the cyanide was cut down to avoid precipitous descent through Stages 3 and 4. When the rat was in Stage 3, the cyanide was cut down still further, and an attempt was made to keep the animal in this stage. Only rarely was it possible to maintain a completely smooth course in Stage 3. More often there were inadvertent dips into Stage 4 or emergence into Stage 2, which were handled by cutting off or increasing the cyanide. During the exposure, the stage of each rat was evaluated constantly, and a running tabulation was kept for future correlations. Severely damaged rats failed to rouse from coma or recovered only after several hours but appeared sluggish and weak. However, many rats with less severe lesions recovered in less than an hour and showed no obvious abnormalities.

Histologic Methods.—Surviving rats were killed with chloroform 24 or 48 hours after exposure, occasionally at other times. The brains were fixed in acetate-buffered 10% formalin, dehydrated in alcohols, briefly infiltrated with Celloidin in methyl benzoate, and infiltrated and embedded in paraffin. Sections cut at 6μ were stained by hematoxylin and eosin and by Luxol Fast Blue, with either cresyl violet or periodic acid-Schiff as counterstain, and in some cases by Bielschowsky method for axis cylinders. In a few cases, frozen sections were stained by oil red O or Sudan IV for neutral fat and by 0.01% toluidine blue for metachromasia. In some experiments the spinal cord, eyes, and other organs were examined.

Results

The procedures described have three advantages over previous methods for producing cyanide lesions of the brain:

1. Lesions are produced in all, or nearly all, survivors of each experiment.

2. Lesions are induced by a single brief exposure (usually 20-45 minutes), in contrast to the multiple injections extending over days, weeks, or months, as used by previous investigators.

3. The site and distribution of the lesions can be predetermined to a certain extent by varying the depth and duration of the intoxication.

The brains of 155 rats with cyanide-induced lesions were available for this study. Of these, 45 had died (or occasionally had been killed) during the first 24 hours after exposure. The remaining 110 rats had been killed 24 hours or longer after exposure. In addition, there were 71 brains of rats that died during or after exposure or were killed after exposure in which no lesions were found. The brains of many of the rats that died during exposure were not studied. These figures should not be taken as an index of the efficiency of the technique, since they include even the earliest experiments involving multiple exposures in a large chamber, as well as experiments in which very short or long and very deep or light exposures were made deliberately.

The cyanide-induced lesions were varied in type and distribution.

Description of Lesions

Corpus Callosum (Figs. 1-5; also Figs. 2 and 3 in Hicks [11]).—The earliest detectable lesions were observed in rats that died or were killed two to five hours after exposure (Fig. 2). There was a fenestration of the callosum due to the appearance of spaces among the fibers. Some of the oligodendroglia nuclei were surrounded by wide clear spaces, but more often the spaces appeared unrelated to glial nuclei. The nuclei of the interfascicular oligodendroglia were slightly shrunken and darker-staining than normal.

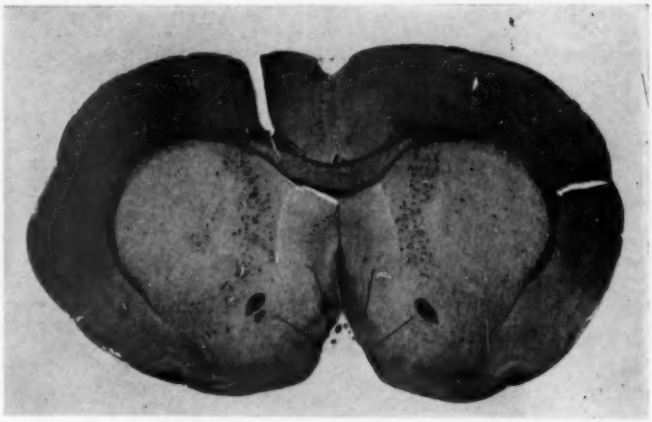

Fig. 1. — Symmetrical demyelination of corpus callosum and corpus striatum one month after a single exposure to hydrogen cyanide. There is preservation of the dorsal and ventral rims of the callosum and the medial bundles of the striatum. Luxol Fast Blue-periodic acid-Schiff-hematoxylin; reduced 25% from mag. × 7.

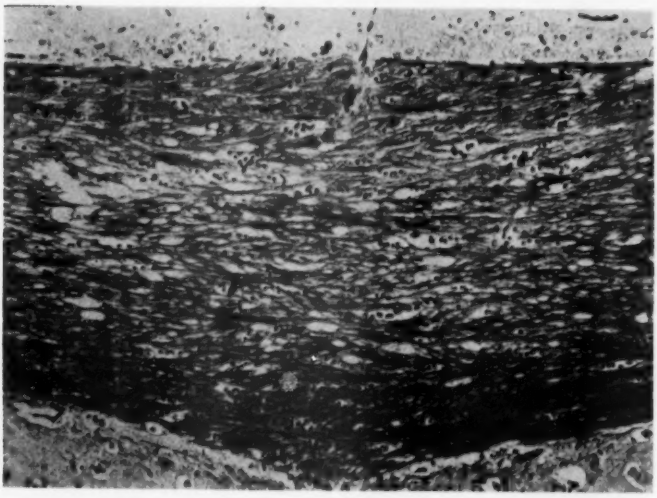

Fig. 2.—Early fenestration and nuclear pyknosis in center of callosum, five hours after exposure. Luxol Fast Blue-cresyl violet; reduced 15% from mag. × 125.

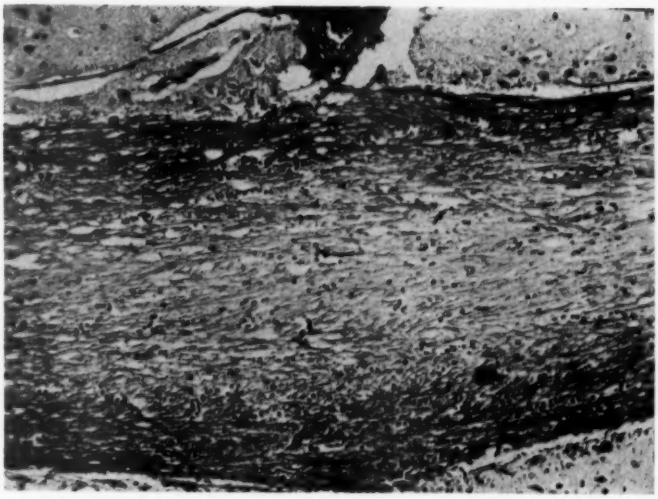

Fig. 3.—Demyelination and advanced pyknosis of interfascicular oligodendroglia in callosum, 24 hours after exposure. Dorsal and ventral rims are spared. Luxol Fast Blue-cresyl violet; reduced approximately 15% from mag. × 125.

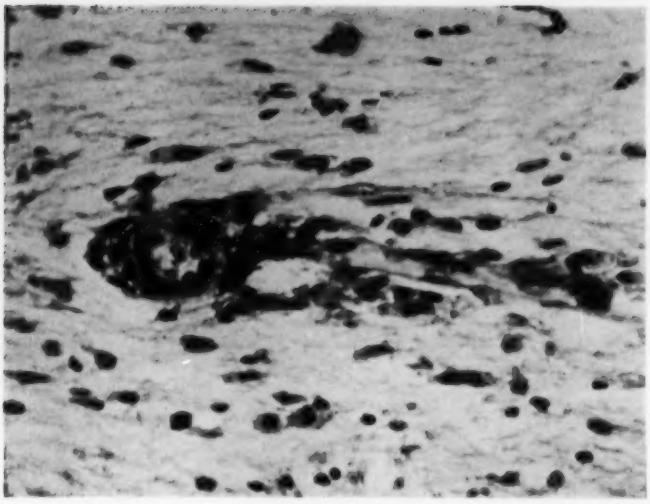

Fig. 4. — Three days after exposure, a callosal arteriole and capillary (branching off horizontally to the right) show increased cellularity, with many phagocytes originating from their walls and extending into the tissue. Hematoxylin and eosin; reduced 15% from mag. × 450.

Twenty-four hours after exposure the lesion was well defined and fairly well demarcated (Fig. 3). The callosal fibers had lost their affinity for myelin stains and also for eosin so the involved area appeared pale. Anteriorly, the entire lesion showed pronounced fenestration; in the bulkier posterior callosum, it was present along all or most of the margins of the lesion. The oligodendroglia nuclei were severely shrunken and pyknotic in both fenestrated and nonfenestrated parts of the lesion. At times a small amount of oligodendroglia cytoplasm was visibly stained with eosin; usually a tail of cytoplasm was visible on one side of the nucleus, tapering off to a point. The pyknotic nuclei were often irregular or angular in shape. Nuclei of astrocytes appeared normal or reduced in number. The vessels were congested. There was no neutral fat in the lesion.

Forty-eight hours after exposure the lesions showed slight hypercellularity, which increased at later times. This was due to

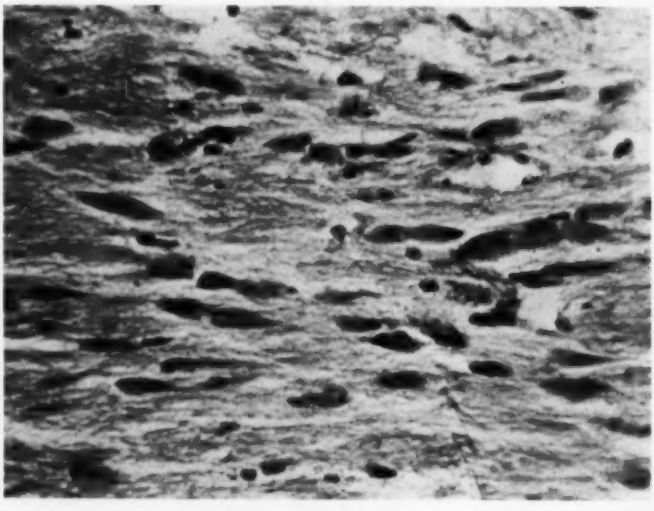

Fig. 5. — One month after exposure, the phagocytes are large and elongated in the axis of the callosum. The cytoplasm is deeply colored by the Schiff reagent. Luxol Fast Blue-periodic acid-Schiff - hematoxylin; reduced 15% from mag. × 450.

persistence of the pyknotic oligodendroglia plus endothelial hyperplasia and the appearance of increasing numbers of macrophages with vacuolated cytoplasm. The macrophages were most numerous at the margins of the lesion and around vessels. The venules and capillaries were prominent, owing to swelling of the endothelial nuclei and increase of cells. Mononuclear cells in the lumen sometimes appeared lined up along the endothelium; emigration through the wall was occasionally suggested, although this is impossible to prove in a fixed preparation. The increased cellularity of the vessel walls, with mitotic activity and with macrophages closely applied on the outside, left no doubt that the vessels were a major source of the macrophages (Fig. 4). Transformation of resting microglia cells provided another source. It was difficult to rule out the possibility that the oligodendroglia might also transform into macrophages.[13] Frequently, rows of pyknotic interfascicular oligodendroglia would have interspersed macrophages. Whether these phagocytes arose from resting microglia or interfascicular oligodendroglia is impossible to say. Spindle-shaped cells were present in increasing numbers after 48 hours; these cells had elongated nuclei, often pyknotic, occasionally in mitosis, with delicate tails of cytoplasm at both ends. The spindle-shaped cells developed into macrophages with vacuolated cytoplasm. Some of these cells became very large, and they were oriented in the direction of the callosal fibers. Polymorphonuclear leukocytes, perivascular cuffing, and vascular thrombosis were not detected. Fenestration became progressively less prominent and was inconspicuous after one week.

One month after exposure, the callosum was grossly shrunken and narrower than normal, with demyelination detectable to the naked eye (Fig. 1). The anterior part of the lesion and the periphery of the posterior part showed pronounced hypercellularity. Nuclear pyknosis was no longer prominent. The astrocyte nuclei were increased in number and size, and many had eosinophilic cytoplasm. The numerous microglial cells had scanty cytoplasm which contained periodic acid-Schiff-positive granules (metachromatic with toluidine blue in frozen section). Macrophages were few. No myelin remained. In contrast, the interior part of the posterior callosal lesion (the part which did not usually show fenestration) still contained some myelin, as indicated by metachromasia and slight staining with Luxol Fast Blue. The area was not highly cellular, but it did contain many very large macrophages whose abundant cytoplasm was metachromatic, contained neutral fat and periodic acid-Schiff-positive material, and in some areas stained with Luxol Fast Blue. The appearance suggested that demyelination proceeded from outside in, and the process of removing degenerating myelin was still active in the center of the bulky posterior callosum even though it was essentially completed elsewhere. Moderate numbers of axis cylinders were preserved in the demyelinated areas.

At no time did cavitation or malacia supervene.

Topography: Severe lesions of the callosum involved almost the entire structure, but there was usually sparing of the anterior and posterior tips and thin dorsal and ventral rims (the ventral preserved rim including the dorsal hippocampal commissure). Rarely a lesion of the posterior half was associated with a slight involvement of the anterior part, the two lesions being separated by a normal area. Often a lesion of the posterior half was accompanied by oligodendroglial pyknosis in the anterior half, without loss of myelin staining. Less severe lesions spared the anterior half completely. The mildest lesions encountered consisted of oligodendroglial pyknosis and/or slight fenestration in the posterior half (rarely in the anterior half). The variation in anteroposterior extent of the lesion was accompanied by a parallel variation in dorsoventral extent. In the severest lesions, the dorsal and ventral rims of unaf-

fected fibers were very thin but were always present.

The lateral extent of the lesions also varied. Most lesions affected the callosum from the midline laterally 1 to 2 mm. to the point of inflection where the callosum with its dorsal concavity joined the callosal radiation with its dorsolateral convexity. The same degree of lateral involvement occurred behind the posterior tip of the callosum, and so sections at the level of the posterior commissure showed lesions in the central white matter of each hemisphere which appeared separate but which were actually continuations of the callosal lesions.

Severe lesions of the entire callosum were sometimes associated with symmetrical extensions far out in the callosal radiation. These extensions went much further in the anterior half (over the corpus striatum) than in the posterior half (over the hippocampus). As was the case for the callosal lesion itself, a dorsal rim was preserved (except in unusually severe lesions), but, unlike the callosal lesion, the ventral rim was often deficient anteriorly over the corpus striatum. The fibers of the hippocampal alveus were almost never involved.

From the foregoing it can be seen that the callosal lesions had a characteristic and reproducible histological appearance, evolution, and distribution. The structures involved can be listed in order of decreasing sensitivity to cyanide: posterior half of callosum, central white matter of hemispheres posterior to the termination of the callosum, anterior half of the callosum, anterior half of callosal radiation, posterior half of callosal radiation.

Corpus Striatum.—The severest lesion of the corpus striatum consisted of necrosis of both gray and white matter (Figs. 6 and 7). Rarely the affected tissue appeared disorganized, with necrosis of vessels and supporting tissue as well as of the parenchyma. More often, the vessels were dilated but not necrotic and the glial cells showed varying degrees of damage, while the neurons and white matter bundles were necrotic. The necrotic neurons showed extreme shrinkage, with marked pallor or with pyknotic nuclei and strongly eosinophilic cytoplasm. The interneuronal background tissue was more eosinophilic than normal tissue and stained pale green rather than pale blue with Luxol Fast Blue. The necrotic white matter was swollen, extremely vacuolated, and pale with pyknotic oligodendroglia and loss of affinity for myelin stains. Less severe lesions involved selective neuronal destruction and demyelination rather than necrosis.

The white matter was often involved selectively in less severe lesions (Figs. 1 and 8). Minimal lesions of this type involved partial loss of affinity for myelin stains

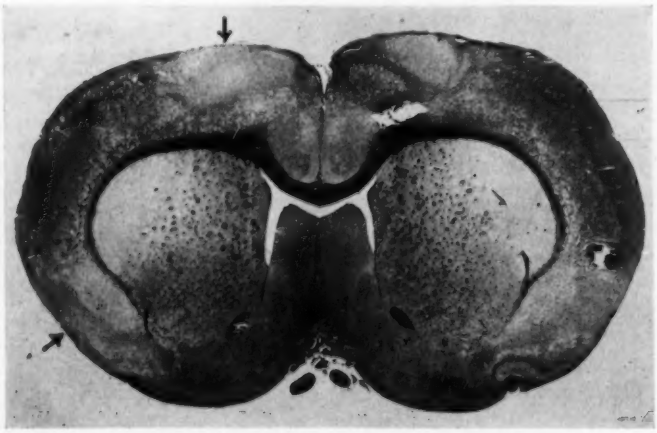

Fig. 6.—Multiple areas of necrosis involving the lateral part of striatum, a dorsal zone of neocortex (arrow), and the pyriform cortex (arrow) from rhinal fissure to region of lateral olfactory tract (which is preserved). Vacuolation is present around all the areas of necrosis, and it also involves the relatively intact lateral neocortex in its deepest layers. The callosum and callosal radiation are intact, despite contiguity with areas of severe necrosis. One day after exposure. Luxol Fast Blue-cresyl violet; reduced 15% from mag. ×7.

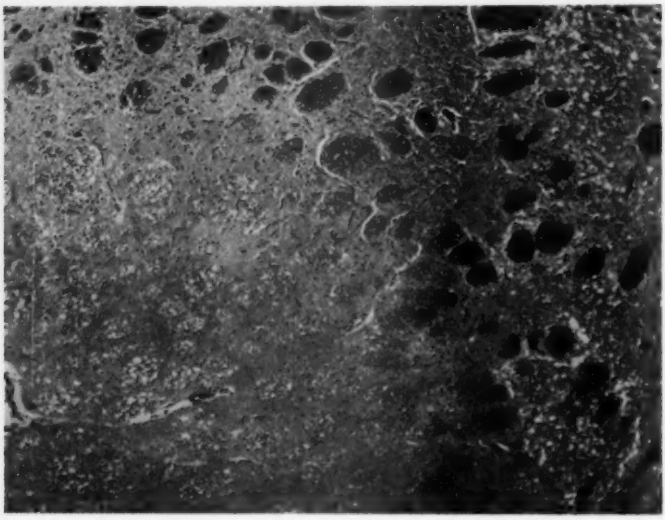

Fig. 7.—Necrosis of the corpus striatum, one day after exposure. The illustration includes the edge of the lesion and shows an area of necrosis of both gray and white matter (left), an intermediate zone in which the myelinated bundles stain poorly and the gray matter is necrotic, and a peripheral zone in which the white matter bundles appear normal but the gray matter is vacuolated (right). Luxol Fast Blue-cresyl violet; reduced 15% from mag. × 50.

but no other abnormalities; these lesions were uncommon. The common demyelinating lesions of the white matter bundles resembled the callosal lesions but were not readily identified before 24 or 48 hours. In the earliest lesions, the bundles appeared pale in hematoxylin and eosin preparations and unstained in myelin preparations. Clear spaces appeared but were not so pronounced as in callosal lesions. The oligodendroglia nuclei were pyknotic.

By 48 hours, each bundle was slightly hypercellular, and this was very marked by 72 hours (Fig. 4 in Hicks[11]). Bundles which normally show 0 to 5 glial nuclei in a given cross section showed 5 to 25 nuclei. These nuclei were small and round; some were pyknotic, and almost all were without stainable cytoplasm. Mitotic figures were readily detected. The blood vessels showed swollen and proliferated endothelial and adventitial cells, as in callosal lesions.

Fig. 8.—One month after exposure, intense hypercellularity of the striatal white matter bundles persists (compare with normal bundles on the right). The gray matter is normal. Hematoxylin and eosin; reduced 15% from mag. × 125.

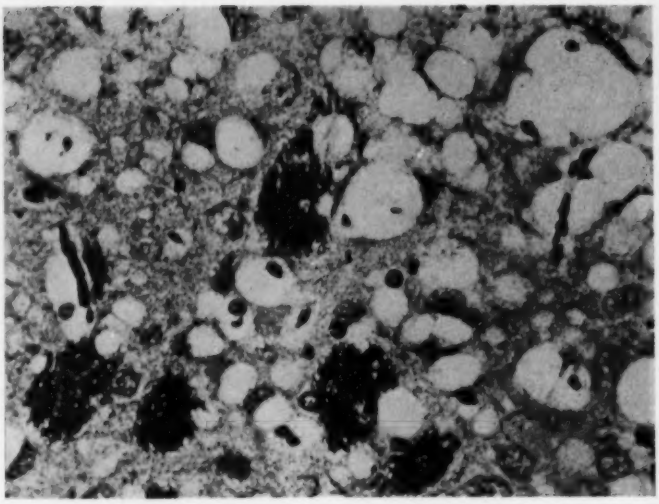

Fig. 9.—Vacuolation of the striatal gray matter, one day after exposure. Clear spaces have appeared in and around neurons and glia, and the pericapillary spaces are dilated. The darkly stained myelinated bundles are spared. Luxol Fast Blue-cresyl violet; reduced 15% from mag. ×450.

Macrophages did not appear so soon and were never numerous; they were observed by 96 hours. The hypercellularity was still marked after one month (Fig. 8); the origin of these cells is uncertain. The axis cylinders were only moderately decreased in number.

In some cases, the gray matter was selectively damaged. Neuronal destruction and disappearance, attended by varying degrees of glial damage, resulted after several weeks in collapse of the gray matter, and so the bundles of white matter came to lie very close to each other. Neuronophagia was not noted. Mitotic activity in glial cells, vascular proliferation, and macrophages were observed.

A frequent lesion of the striatum consisted of patchy vacuolation of the gray matter (Fig. 9). The neurons and glia were distended by large clear spaces, with consequent distortion of their shapes. Often there was only a thin rim of cytoplasm or a compressed nucleus at the periphery of a vacuole, but this served to indicate the intracellular location of the vacuole. Often, however, the vacuoles appeared unrelated to cells. Even minor degrees of vacuolation were accompanied by vascular endothelial swelling and proliferation, mitotic activity in glia, and widening of the perivascular spaces.

Topography: The lesions appeared round or oval and moderately well demarcated and were usually, but not always, bilateral (Fig. 1). In frontal sections typical lesions of the striatum—whether of gray matter, white matter, or both—involved the central and lateral or ventrolateral portions. All but the severest lesions spared a rim of gray and white matter along the dorsal and medial borders. The lateral and ventral borders were less often spared or were spared only to the extent of a very thin rim. Minimal lesions always occurred in the anterior portion (at the level of or anterior to the front tip of the septum); moderate lesions involved the middle portion as well (level of the septum), while only the severest lesions involved the posterior portion (level of globus pallidus) or the extreme anterior tip.

Zones of severe necrosis of gray and white matter were surrounded by zones of less damage, usually selective neuronal loss and/or varying degrees of damage to white matter. These, in turn, were often surrounded by zones of vacuolation of the gray matter (Fig. 7).

Vacuolation of striatal gray matter, in addition to its occurrence on the periphery

of severer lesions, also occurred in irregular patchy distribution in an otherwise normal striatum. Hicks [14] described a similar change in the rats and mice treated with methionine sulfoximine, and he considered the possibility that it ". . . could represent a still reversible state of damage. . . ." Our observations support this possibility because the vacuolation occurred on the margins of lesions which showed an obvious gradation of severity from center to periphery and because vacuolation appeared to represent a transitory abnormality, rarely observed after 48 hours.

When the striatal lesion was severe, the overlying callosal radiation occasionally showed a few pyknotic oligodendroglia and pale fibers, regardless of the absence of a lesion in the callosum itself. Conversely, and more commonly, a severe callosal lesion extending out into the callosal radiation was associated with necrosis of gray and/or white matter in a strip of contiguous underlying dorsolateral striatum. This was observed both with and without an independent lesion of the striatum in the usual central and ventrolateral location.

Pyriform Cortex.—Lesions detected early (two to five hours after exposure) exhibited pale-staining and clear perineuronal spaces due to cell shrinkage. After 24 hours, lesions appeared as bilateral or unilateral zones of necrosis in which the neurons were destroyed, the neuropil altered in staining reaction, and the glia damaged. A peripheral zone of vacuolation of neurons and neuropil was often noted and sometimes extended into the neocortex but only in the deepest layer (Figs. 6 and 10).

Minimal lesions appeared as small focal zones of necrosis or neuronal necrobiosis in the molecular or underlying layers, especially in the posterior part of the hemisphere. Lesions of intermediate severity were localized to the region of the rhinal fissure. Severe lesions caused destruction of the entire pyriform cortex from the dorsal lip of the rhinal fissure ventrally to the region of the lateral olfactory tract (which was never involved). At more posterior levels, the involved area extended to the ventral surface of the hemisphere and to the amygdaloid nuclei. Lesions of the pyriform cortex often appeared to be continuous with associated lesions of the corpus striatum, but frequently a few preserved fibers of the callosal radiation separated the two necrotic structures.

Neocortex.—Lesions of neocortex resembled those of pyriform cortex (Figs. 6 and 10). Minimal lesions were focal areas of necrosis involving the upper cortical

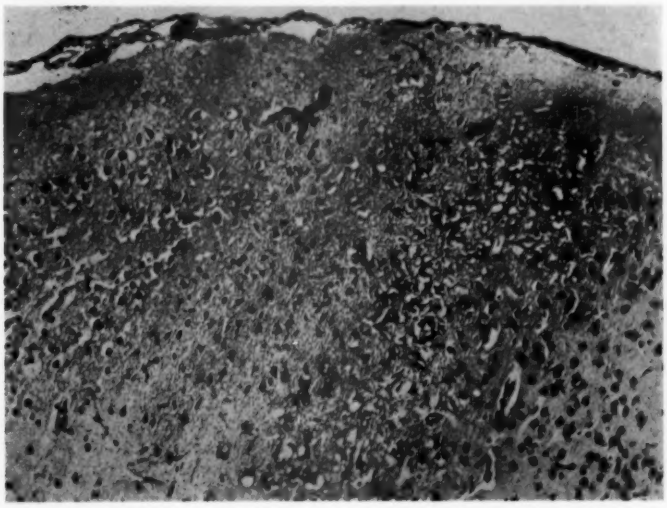

Fig. 10.—The margin of a lesion of the pyriform cortex, one day after exposure. The shrunken neurons in the necrotic zone on the left are separated from the uninvolved area (lower right corner) by a slanting zone with marked vacuolation. Hematoxylin and eosin; reduced 15% from mag. × 125.

Fig. 11.—A lesion of the neocortex, one day after exposure. In addition to shrunken, necrotic neurons there are parallel chains of vacuoles oriented at right angles to the surface. Hematoxylin and eosin; reduced approximately 15% from mag. × 125.

layers, usually dorsal, and often extending as a longitudinal strip along the length of the hemisphere. Severe lesions exhibited multiple patchy areas of necrosis or, more commonly, necrosis of the entire neocortex of one or both hemispheres. Frequently the cingular cortex was spared. Necrotic areas usually involved all layers, with relative sparing of the molecular layer and occasional sparing of the deepest layer. Vacuolation was present at the periphery, in the adjacent non-necrotic areas, and within the lesion itself, where it was sometimes so severe as to give the cortex a "soap bubble" appearance. Parallel chains of vacuoles, oriented at right angles to the surface, suggested that the vacuolation might be related to neuronal or glial processes (Fig. 11).

Hippocampus.—A few rats showed destruction of the entire hippocampus, but most of the lesions consisted of neuronal necrosis of the dorsal pyramidal-cell layer,

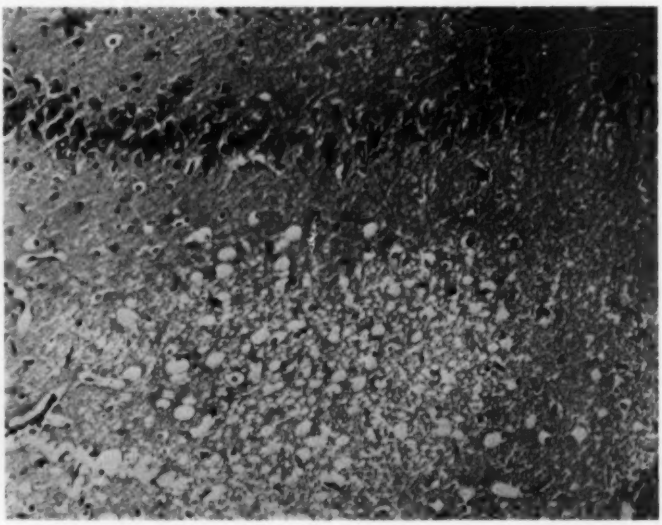

Fig. 12.—A lesion of the hippocampus, one day after exposure. The pyramidal layer shows the contrast between intact (left) and shrunken, necrotic (right) neurons. The stratum radiata shows an area of vacuolation. Hematoxylin and eosin; reduced 15% from mag. × 125.

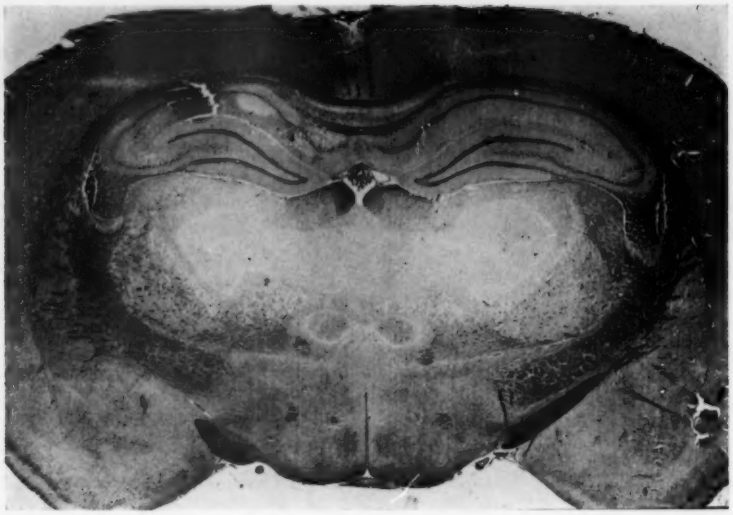

Fig. 13.—The thalamus shows a pale, symmetrical, butterfly-shaped area of necrosis, one day after exposure. Lesions are also present in the callosum and in the hippocampus (left). Luxol Fast Blue-cresyl violet; reduced 30% from mag. × 11.

especially the medial portion at anterior levels. Only 4 of the 18 rats that survived 24 hours with hippocampal lesions had, in addition, necrosis of fascia dentata neurons, and, here again, the dorsal lamina was most susceptible. Patchy vacuolation of the stratum radiatum and stratum oriens and demyelinization of the alveus were occasionally seen in severe lesions (Fig. 12).

Cerebellum.—Necrobiosis of Purkinje cells was observed to involve isolated cells, small groups, or entire folia. Vacuolation of the affected area and enlargement of the Bergmann glia were observed. The granular layer was involved in only one rat.

Substantia Nigra.—The lesions were focal, involving the neurons and/or surrounding white matter. (They appeared identical with those produced by Hicks with asphyxia in nitrogen and illustrated in his Fig. 7 [11]).

Diencephalon.—The lesions were symmetrical butterfly-shaped zones of necrosis involving the medial, ventral, and lateral thalamic nuclei, joined across the midline through the massa intermedia (Fig. 13). Most of these lesions occurred in severely damaged animals, which died within a few hours after exposure, but a few rats lived 24 hours.

Anterior Commissure.—The lesions resembled those of the callosum. They occurred only in the midline, appeared to involve the temporal division of the commissure preferentially, and spared the dorsal and ventral rims (Fig. 14). These lesions are easily missed if the sections do not show the commissure as it crosses the midline.

Ventral Hippocampal Commissure.—The lesions also occurred in the midline but sometimes extended laterally into the fimbria. Fenestration was sometimes extremely pronounced (Fig. 15).

Optic Nerves, Chiasm, and Tracts.—These structures were sometimes involved separately or together with mild vacuolation. The cerebral peduncle occasionally showed a similar change.

Factors Influencing the Occurrence and Distribution of Lesions

Size of Rat.—Distinct differences between large (200-300 gm.) and small (100-200 gm.) rats were noted as to both incidence and distribution of lesions. Of 64 large rats that survived 24 hours, only 11 (17%) had

Fig. 14.—The anterior commissure shows demyelination and hypercellularity, three days after exposure. The dorsal and ventral rims are preserved, just as in the case of callosal lesions. Luxol Fast Blue-cresyl violet; reduced 15% from mag. × 50.

no brain lesions. Of 108 small rats that survived 24 hours, 51 (47%) had no lesions.

The corpus callosum was the structure most frequently affected in large rats; the striatum was never involved alone but only in conjunction with callosal lesions (Fig. 1); the cortex was involved infrequently. In contrast, the striatum was the structure most frequently affected in small rats, and it was very often the sole site of involvement; the callosum was injured in far fewer animals; necrosis of the cortex was frequent (Fig. 6).

Depth of Intoxication.—Only rarely was it possible to keep the rat in the desired state for the entire duration of an exposure. Usually there were inadvertent changes to deeper or lighter levels of intoxication. Nevertheless, after many experiments, the incidence of lesions could be correlated with the depth of intoxication.

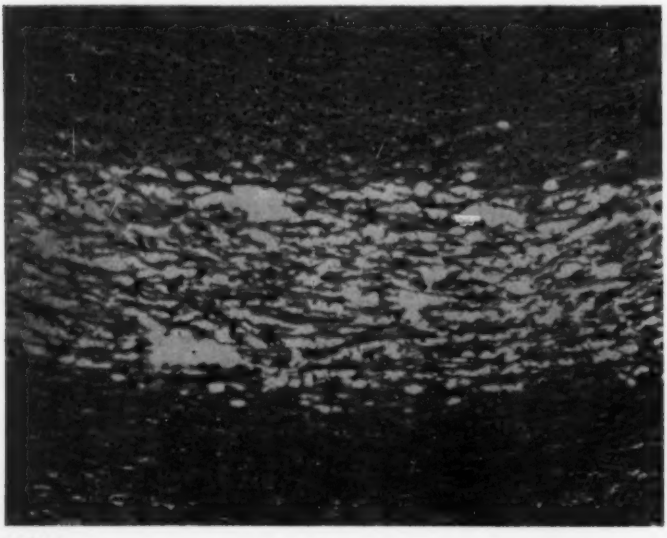

Fig. 15.—A lesion of the ventral hippocampal commissure, one day after exposure, showing oligodendroglial pyknosis, midline location, and preservation of dorsal and ventral rims, just as in the case of callosal lesions. However, the fenestration is unusually pronounced. Hematoxylin and eosin; reduced 15% from mag. × 125.

Exposure in Stages 1 or 2 was ineffective in producing brain lesions. For example, seven rats were kept in Stages 1 and 2 for two hours and the exposure repeated up to six times on subsequent days. Only one rat developed a lesion (white matter of striatum), despite the lengthy and repeated exposures.

Exposure in Stage 3 and/or 4 was effective in producing brain lesions. Stage 3 was sufficiently deep to produce lesions of callosum and basal ganglia. However, lesions of the neocortex were never produced and lesions of the hippocampus and other areas of gray matter were rarely produced unless the exposure reached Stage 4 for at least a few minutes. Most exposures involved a mixture of Stages 3 and 4. When Stage 4 predominated, especially when the intoxication was sufficiently profound to cause respiratory depression, there was a high incidence of deaths during exposure or within the next few hours. Lesions were detected histologically very early (two hours or less) after exposure and revealed predominant damage to gray matter (cortex, thalamus, hippocampus, striatum, and cerebellum) in these cases.

Duration of Exposure.—The minimum time for induction of lesions was difficult to determine because of the difficulty in maintaining a given stage and because the cyanide effect persisted for a variable time after administration was stopped. Less than 10 or 15 minutes of exposure in Stage 3 (not including the period of persisting effect) was ineffective in producing lesions. Fifteen to thirty minutes of continuous Stage 3 exposure was usually adequate to injure the callosum and possibly other areas of large rats. More than 30 minutes of Stage 3 generally constituted a severe exposure, with the possibility of injuring multiple structures or causing early death. The small rats required longer and deeper exposures, resulting in a higher incidence of early deaths and more damage to gray matter.

Periodicity of Exposure.—A brief return to Stage 1 or 2 appeared to interfere with the cyanide effect, and so exposures which seemed to be adequate in depth and duration sometimes failed to cause lesions. On the other hand, brief returns to Stage 1 or 2 appeared to improve the ability of some of the rats to live through prolonged exposures and to survive with lesions in relatively unusual areas—for example, the anterior commissure.

Association of Lesions.—The lesions in 110 rats (large and small) that survived 24 hours are classified in the Table according to whether they occurred alone or in as-

Classification of Lesions *

Structure with Lesion	Rats, No.	Associated Lesions												
		None	Callosum	Striatum (White)	Striatum (Gray)	Pyriform Cortex	Neocortex	Hippocampus	Purkinje Cells	Thalamus	Nigra	Ant. Comm.	V. Hippoc. Comm.	Optic Pathway
Callosum	65	23		19	12	7	1	7	5	2	2	4	6	5
Striatum white	34	10	19			3	0	4	2	1	1	2	1	3
Striatum gray	27	4	12			16	7	5	3	1	0	1	1	1
Pyriform cortex	22	1	7	3	16		8	8	3	0	3	0	0	0
Neocortex	11	1	1	0	7	8		6	1	0	1	0	0	0
Hippocampus	18	2	7	4	4	8	4		1	2	3	0	0	0

* The incidence of lesions in certain structures of 110 rats that survived hydrogen cyanide exposure at least 24 hours and the incidence of associated lesions in other structures. The figures for striatum (gray) include cases in which the white matter was damaged simultaneously. The selectively demyelinating lesions of the striatum are listed under "Striatum (White)". Vacuolation of the gray matter of the striatum has not been included in this tabulation. The number of rats with lesions in a given structure is always less than the sum of the figures for associated lesions because some rats had several associated lesions.

sociation with other lesions. The Table reveals that the callosum and the striatum (white matter) were the only structures frequently involved in solitary lesions. In contrast, lesions of gray matter (striatum gray matter, pyriform cortex, neocortex, hippocampus) rarely appeared as isolated lesions but much more often in groups. Especially frequent was the association between striatum gray matter and pyriform cortex. These facts, in conjunction with the data on depth of intoxication, are taken to indicate that the areas of white matter under discussion are more susceptible to cyanide than the areas of gray matter under discussion. The Table also reveals that seldom-involved areas of white matter (anterior commissure, ventral hippocampal commissure, optic nerves, chiasm, and tracts) were damaged only in conjunction with the commonly involved areas of white matter.

The figures in the Table clearly indicate the tendencies to association of certain lesions, but they cannot be taken to indicate the relative frequency of lesions, since large and small animals have been tabulated together. Deaths in less than 24 hours have not been included, since many such cases were subject to autolysis and to the possibility that the lesions were not fully developed; their inclusion would increase the incidence of lesions in gray matter, since they represent the severer exposures.

Also, the association of certain lesions does not imply similar severity. For example, severe lesions of the striatum were associated with both severe and mild lesions of the pyriform cortex.

Individual Variation.—This factor was partly eliminated by substituting individual jars for the large chamber, but it still presented a problem. Often, rats subjected to apparently similar exposures developed lesions of differing severity or in different areas. One explanation may be local or systemic differences in ability to detoxify cyanide. To illustrate the importance of the detoxification mechanism, 1 gm. of sodium thiosulfate was administered to rats intraperitoneally, subcutaneously, or by gastric tube. It caused great increase of resistance to hydrogen cyanide by accelerating the detoxification.

Comment

The results have demonstrated that exposure of rats to hydrogen cyanide in individual chambers constitutes a reliable and practical technique for producing brain lesions. We have had most success in obtaining callosal lesions at will. For this purpose, 250 gm. rats should be exposed for 20-30 minutes in Stage 3, with as little Stage 4 exposure as possible. With experience, the mortality can be kept down to zero to two of six rats, and all or almost all of the survivors will have callosal lesions, with or without lesions in other areas. Lesions in the striatum, cortex, and elsewhere can also be produced, especially in smaller animals, but with less certainty and at the expense of greater initial mortality.

It has generally been assumed that the mechanism of cyanide action involves its inhibition of the cytochrome oxidase system and consequently of cellular oxidation. This inhibition is reversible, as indicated by the failure of brief exposures, even in Stage 3 or 4, to cause lesions. Windham [15] found that the inhibition was reversible no matter how long the cyanide treatment was continued. What happens when the reversible inhibition turns into an irreversible destructive lesion? It seems likely that cells cannot tolerate inhibition of oxidative processes indefinitely and that after a certain period the cell can no longer maintain its structural integrity. Death or at least severe disorganization of the cell ensues.

The neuron is sensitive to cyanide because of its high metabolic demands. We have demonstrated neuronal necrosis in cortex, striatum, hippocampus, cerebellum, thalamus, and substantia nigra. The vacuolation of neurons may represent a severe disorganization of the cell, possibly just short of necrosis.

White matter (callosum and striatum) is more sensitive to cyanide than gray matter. This is thought to be due to its low content

of cytochrome oxidase. It is possible that necrosis or profound disorganization of the oligodendroglia constitutes the key to these lesions. Certainly nuclear pyknosis and shrinkage of the interfascicular oligodendroglia were most conspicuous and early changes in callosal lesions. The fenestration which occurred early appeared to be between rather than in myelinated fibers; further studies will be made to ascertain whether the fenestration is related to glial processes. The demyelination may be explained on the same basis if it is accepted that oligodendroglia are responsible for myelin maintenance.

One of the striking things about cyanide encephalopathy is the multiplicity of sites involved in both gray and white matter. Nevertheless, the lesions are not universal in distribution; the cord and most of the brain stem, cerebellum, and diencephalon and part of the cerebrum were spared. Even in areas of involvement, there was a gradient of sensitivity to cyanide in adjacent parts of the same structure. An acceptable theory of cyanide action must explain the differential sensitivity of different parts of the brain and of adjacent parts of the same structure as well as the differences between large and small animals. It is not certain whether all these features can be explained by variations in metabolic demand or cytochrome oxidase content or by the relation between these two factors, and this problem is currently under investigation. Another important variable may be the small fraction of cellular metabolism which is insensitive to cyanide and would tend to preserve the cell. In other words, there may be qualitative or quantitative differences in metabolic pathways in different areas of the brain.

The differences in results in small and large animals serve to emphasize that the nervous system changes character even after myelin stains show an "adult" picture. This has been confirmed by chemical analyses and response to other demyelinating agents.[16]

Jervis [4] has pointed to vascular factors which may influence the distribution of cyanide lesions: richness of capillary network, effect on vasomotor innervation with resulting vasoparesis or vasospasm, paralysis of circulatory center, toxic effect on heart. We have noted occasional unilateral lesions in cortex and striatum. Perhaps a circulatory factor is responsible for this phenomenon. If the circulation to one hemisphere is inherently low, or if it is decreased by a mechanical factor like the position of the head or neck, the ischemia may contribute to cyanide sensitivity. (Experiments on this problem are in progress.)

Histotoxic anoxia (cyanide) differs from anoxic anoxia in that the arterial blood is normally saturated with oxygen in the former condition. However, respiratory depression is a feature of Stage 4 cyanide intoxication. The possibility remains that the cortical lesions characteristic of Stage 4 exposures are due to the effects of histotoxic anoxia combined with anoxic anoxia or with some other factor, but this remains to be proved.

We have no evidence to indicate that either ischemia or anoxic anoxia contribute to the demyelinating lesions of the callosum, etc. Neither do we have any evidence that cyanide causes demyelination through an indirect or secondary mechanism, as suggested by Hurst [17] and by Lumsden ("It may be, therefore, that the demyelination is a secondary phenomenon, sui generis; that the toxic or lytic agent which ultimately does the damage to the white substance is not the cyanide itself but one derived and spreading from a central focus within the white matter.")[9] The fact that we have produced with single treatments callosal lesions apparently identical to those produced by other authors with multiple treatments suggests that the cyanide acts directly, and it is not necessary to invoke the secondary mechanism of a "myelinolytic ferment."[9]

Summary

Rats, after a single brief exposure to hydrogen cyanide vapor by a technique that permits control of the depth and duration of

intoxication, develop lesions in any of several areas of gray and white matter. Lesions of the gray matter may involve neocortex, pyriform cortex, corpus striatum, hippocampus, cerebellum, nigra, and diencephalon, and they tend to occur in groups and to follow very severe cyanide poisoning. Lesions of the white matter follow less severe cyanide poisoning and may involve one or more of the following sites: corpus callosum, corpus striatum, ventral hippocampal commissure, anterior commissure, optic nerves, and chiasm. The experimental conditions can be controlled so as to produce lesions of the corpus callosum in a regular and reproducible fashion. These results with cyanide emphasize that the presence or absence of histopathologic effects and the distribution of lesions depend in a very critical way on the size of the animal and on the careful control of depth and duration of intoxication.

Addendum

Several rats were killed four months after exposure to hydrogen cyanide, and the following features were observed. The lesions of the corpus callosum were greatly decreased in cellularity, and only a few macrophages remained. The lesions varied from narrow, inconspicuous glial scars to broad bands of fibrillary glial tissue containing a few reticulin fibers. The callosum was shrunken. The demyelinated zone often extended into the callosal radiation.

The lesions in the corpus striatum were of all degrees of severity. In the mildest lesions, the hematoxylin and eosin stain revealed no abnormality, but the Luxol Fast Blue preparation showed partial-to-complete demyelination of the central white matter bundles. Close inspection showed that the affected bundles contained a few glial cells whose cytoplasm was colored by the periodic acid-Schiff counterstain. In severer lesions the gray matter of part of the striatum has disappeared, and, as a result, the white matter bundles were in close apposition. In the severest lesions, both gray and white matter had disappeared from part of the striatum, leaving a shrunken, poorly demarcated scar containing numerous vessels and astrocytic nuclei. The apparent increase of vessels and astrocytes was at least partly owing to the collapse of tissue and loss of substance. The loss of brain substance was also reflected in the dilatation (*ex vacuo*) of the ventricles.

One rat showed bilateral destruction of the hippocampus including alveus and dorsal hippocampal commissure, with sparing only of part of the dentate fascia. The destroyed tissue was represented by a narrow band of glial cells and vessels with a few phagocytes. The fimbriae and ventral hippocampal commissure were atrophic and partly demyelinated, and the ventricles, dilated (*ex vacuo*).

In summary, destructive lesions of callosum, striatum, and hippocampus, despite extensive loss of tissue, resulted in shrunken scars rather than malacic cysts.

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Further Histochemical Studies of Esterases by 5-Bromoindoxyl Acetate

I. Reference to Nonspecific and Nonenzymatic Hydrolysis

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Indoxyl acetate has several advantages over the existing substrates for the histochemical studies of esterases in tissue. These advantages are, first, that the reaction is "single phase," where a soluble colorless substrate will by the action of the enzyme become a highly insoluble and colored compound. Second, the final reaction product is fine and uniform and has little tendency to crystal formation. This is particularly true in the halogen-substituted indoxyl esters. Third, the resulting product is insoluble in lipid solvents and can be cleared in alcohols and xylene. These advantages are clearly apparent and are consistent with some of the major substrate requirements laid down by Pearse.[1] The indoxyl acetate is not specific but is hydrolyzed by a number of esterases conventionally referred to as nonspecific esterase, lipase, and specific and nonspecific cholinesterase.

The principle involved in both the halogen-substituted and the nonsubstituted indoxyls is the same and can be illustrated by 5-bromoindoxyl acetate. The esterase in tissue sections hydrolyzes the acetate, forming a transient compound, 5-bromoindoxyl, which is readily oxidized to form the highly insoluble and chromogenic compound, 5,5′-dibromoindigo at the sites of enzymic activity. This principle was first established with the unsubstituted indoxyl ester.[2,3]

The unsubstituted indoxyl ester gives, however, a final reaction product which is crystalline in character and tends to obscure the precise localization of the enzymes in tissue. This can be alleviated by the substitution of bromine in the five position of the indoxyl ester. The results accruing from the use of this compound, viz., 5-bromoindoxyl acetate, give a far superior final reaction product and have been described by Holt [4] and by Pearson and Defendi.[5] This final reaction product is extremely fine, noncrystalline, and uniform, affording precise localization. When this substrate was compared in our laboratory with α-naphthyl acetate and naphthol AS acetate it was found that the 5-bromoindoxyl acetate worked well at all ranges of pH from 4.8 to 8.4 without extracellular diffusion of the final reaction products or other artifacts.[5] It obviated the protein-naphthol binding of the other substrates, which, as Defendi [6] has shown in our laboratory, must be taken into consideration when these substrates are used.

Because of the superiority of 5-bromoindoxyl acetate it was decided that further studies of esterases in tissues should be undertaken. The objectives of this study were to determine the effect of pretreatment of tissue sections by solvents in the extreme cold on the enzyme activity,[7] the influence of added ions, the effect of formalin fixation, and the nonenzymatic reaction for imidazole groups.

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Detroit Institute of Cancer Research and Department of Pathology, Wayne State University College of Medicine.

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Materials and Methods

Preparation of Tissue.—About 150 rats were used for this study. Immediately after they were killed small blocks of tissue 2-3 mm. were quickly frozen at −70 C and transferred to a Linderström-Lang cryostat and cut at −20 C and 5μ. The tissue was affixed to slides and stored at −25 C until ready for use.

Incubating Solutions.—The following standard incubating solutions were used, with minor modification which will be referred to under appropriate headings.

Standard Solution I:
- 8 ml. tris (tris[hydroxymethyl]aminomethane) buffer, 0.1 M, pH 7.19
- 3 ml. potassium ferricyanide, 0.05 M
- 3 ml. potassium ferrocyanide, 0.05 M
- 1 ml. 5-bromoindoxyl acetate, 5 mg. per milliliter (ethanol solution)

Standard Solution II:
- 8 ml. Walpole's acetate buffer, 0.1 M, pH 5
- 3 ml. potassium ferricyanide, 0.05 M
- 3 ml. potassium ferrocyanide, 0.05 M
- 1 ml. 5-bromoindoxyl acetate, 5 mg. per milliliter (ethanol solution)

Standard Solution III:
- 10 ml. tris buffer, 0.2 M, pH 8.1
- 5 ml. copper sulfate, 10^{-5} M
- 1 ml. 5-bromoindoxyl acetate, 5 mg. per milliliter (ethanol solution)

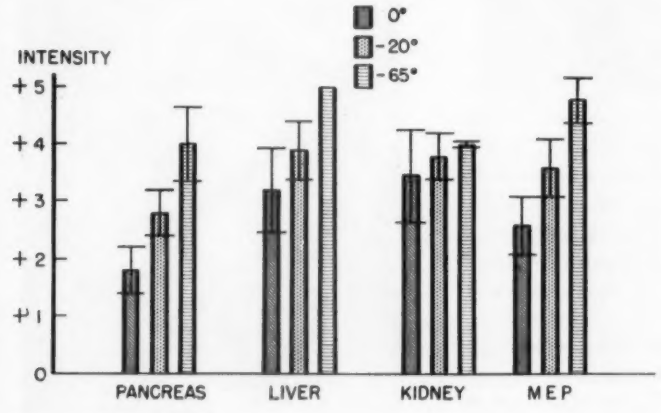
Standard Solution IV:
- 15 ml. tris buffer, 0.1 M, pH 7.19
- 1 ml. 5-bromoindoxyl acetate, 5 mg. per milliliter (ethanol solution)

Standard Solution I was used for the motor end-plates, with 10 minutes' incubation time. Solution II was used for kidney, liver, and pancreas, with 15 minutes' incubation, and for adrenal and small intestine, with 30 minutes' incubation. Solution III was used for the brain, with 30 minutes' incubation time. Solution IV was used for blood and bone marrow smears, thrombi, spleen, lymph node, and thymus, with 30 minutes' incubation.

Pretreatment Procedure.—The stored slides with the affixed tissue were placed in a staining rack and immersed immediately in a precooled pretreatment solution at −65 C, consisting of various organic solvents combined volume to volume in a ratio of 1:1. These solvents were *n*-butyl alcohol (*n*-butanol) (B), petroleum ether (E), chloroform (C), acetone (A), ethyl alcohol (ethanol) (Et), methyl alcohol (methanol) (M), carbon bisulfide (CS_2), and dimethylformamide (F). The solvent mixtures that were used were B/E, B/C/E, B/C/A, B/E/A, C/E/A, Et/C, M/Et/C, M/C, CS_2, and F. After remaining in the solvent mixture for 20 to 50 minutes, the staining rack containing the slides was removed and immediately washed in water with agitation with three rinses, one to two minutes each. The slides were then placed in Coplin jars containing the incubation solution.

Results

Effect of Pretreatment.—Four tissues were used, viz., pancreas, liver, kidney, and motor end-plates. These tissues were pretreated at 0 C, −20 C, and −65 C for 30 minutes. The slides were incubated in the standard solutions optimal for the organs involved. The resulting 5,5′-dibromoindigo representing enzymic loci was graded as visual intensities, which were plotted with their standard deviations, as seen in the Chart, showing the compiled results of all mixtures. The results indicate that the esterase reaction is diminished slightly with



Mean visual intensities of 5,5′-dibromoindigo plotted on ordinate. The standard deviations are represented as barred graphs. The effect of temperature can be seen in the pancreas, liver, and motor end-plates (*MEP*).

increases in temperature in the pancreas, liver, and motor end-plates. No significant change could be seen in the kidney. The effect of temperature on esterase is different from that seen in succinic dehydrogenase, as this was destroyed entirely at 0 C.[7]

Effect of Ions.—In determining the effect of ions on esterase activity, 5 ml. of the following salts in solutions was added to the standard incubation solutions for the various organs. These were $Fe_2(SO_4)_3$, $FeSO_4$, $Co(NO_3)_2$, $CdCl_2$, KNO_3, $CaCl_2$, NaCl, $Mg(NO_3)_2$, and $Al_2(SO)_3$, all at 0.025 M, and $CuSO_4$ at 10^{-5} M.

The ionic effects were not very striking except in esterases of the central nervous system and the motor end-plates. No effects could be seen in the liver and the pancreas. Certain ions inhibited the reaction completely both in the brain and the motor end-plates. These were ferric, cadmium, and aluminum ions, and in addition ferrous ion inhibited the reaction in the motor end-plates.

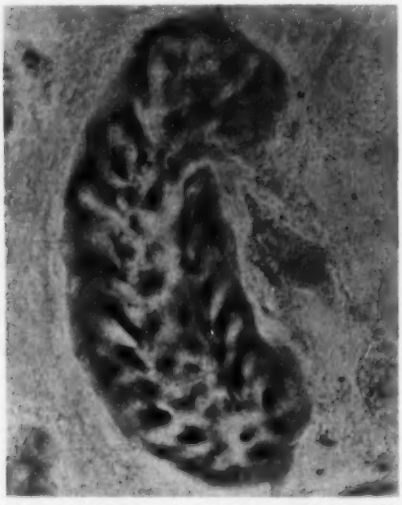
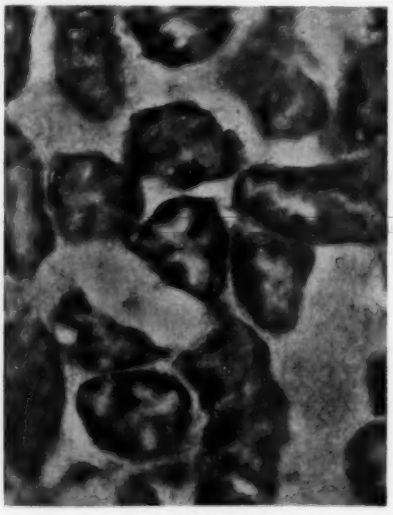


Fig. 1.—Kidney, showing proximal convoluted tubule. Fixed in 1% calcium chloride and neutral formalin for 30 minutes at 5 C. Intense loci of 5-bromoindoxyl esterase activity are seen in the cytoplasm; × 400.

The additive effects of ions become important in determining intracellular localization of enzymic sites. These effects can be illustrated as follows. If a slide is fixed in a 1% solution of $CaCl_2$ in neutral formalin for 30 minutes at 5 C and then incubated in 5-bromoindoxyl acetate, one sees loci of intense enzymic activity, described as

Fig. 2.—Same as Figure 1, except that it is fixed in 1% magnesium chloride and neutral formalin for 30 minutes at 5 C. The 5-bromoindoxyl esterase activity is intense, involving perinuclear and basilar areas of the cell; × 400.

Fig. 3.—Same as Figure 1, except that it is unfixed but pretreated with C/E/A at −65 C for 30 minutes. The 5-bromoindoxyl esterase activity is in the basilar portion of cell with crescent formations; × 400.



"spherical inclusion bodies" by Holt [8] (Fig. 1). However, when magnesium ions are added to the fixative, marked perinuclear enzyme activity is seen (Fig. 2). In neither of these two cases were lipids removed by pretreatment at −65 C. In pathologic material it may become necessary to remove lipids, which may interfere with the reaction. When kidney is pretreated with C/E/A for 30 minutes at −65 C and no ions added, the final reaction product is fine and particulate, as seen in Figure 3. In none of these cases was there actual loss of enzymic activity due either to ion effect or the effect of pretreatment, nor was there any extracellular diffusion of the enzyme or the final reaction product. It is difficult for us to decide whether or not there is more or less intracellular diffusion in one case than another. We feel that in pathologic material where lipids must be removed in order not to interfere with the final reaction the use of pretreatment gives best preparation.

Effect of Formalin Fixation.—To determine the effect of formalin fixation 5μ sections of liver, kidney, motor end-plates, brain cortex, and pancreas were used. Formalin fixation varied (1, 2, 4, 8, and 24 hours) for each tissue, with a sufficient number of samples for each time interval. The normal visual intensity of the final reaction product was established for all the organs. The "half-life," as expressed by the time in which the reaction was one-half of the normal reaction without formalin fixation, varied in the different organs. As thus expressed, the "half-life" for enzyme in the liver was 8 hours; kidney, 12 hours; brain cortex, 4 hours; motor end-plates, 2 hours, and pancreas, 1 hour. However, any formalin fixation over one hour destroys enzyme rapidly and should be avoided.

Effect of Imidazole Groups.—In 1957, Bruice and Schmir [9] showed that imidazole was an effective basic catalyst for the hydrolysis of phenyl acetate. Since phenyl acetate resembles structurally indoxyl acetate, experiments were conducted to determine whether imidazole or related compounds would hydrolyze 5-bromoindoxyl acetate. Five compounds containing imidazole groups were tested, namely, imidazole, histamine, histidine dichloride, histidine monochloride, and benzimidazole. The reaction was run with 5-bromoindoxyl acetate at pH 7.2 for 30 minutes' incubation at 25 C at concentrations of 0.1 M, 0.01 M, 0.001 M, and 0.0001 M. Imidazole gave a strong reaction beginning at five minutes and histamine less reaction (Table). Also, 1% solutions of trypsin, chymotrypsin, and lipase were tested, with positive results in the latter two. This indicates to us that the reaction is less specific than formerly thought, as it is positive with chymotrypsin as well as with nonenzymatic substances having imidazole groups.

Reaction of 5-Bromoindoxyl Acetate with Imidazole Groups at pH 7.2 and 30-Minute Incubation at 25 C

	Concentration			
	0.1 M	0.01 M	0.001 M	0.0001 M
Imidazole	++++	+	--	--
Histamine	++	--	--	--
Histidine dichloride	--	--	--	--
Histidine monochloride	--	--	--	--
Benzimidazole	--	--	--	--

Histochemical Descriptions

Kidneys.—The 5-bromoindoxyl esterase reaction was localized in the cortex, particularly in the convoluted tubules. The interstitial tissue, glomeruli, vessels, and collecting tubules were negative. The pH optimum for this reaction was 5, and the incubation time, 15 minutes. The fine detail of the intracellular distribution can best be appreciated in the convoluted tubules. With pretreatment and without added ions intense enzyme activity was seen, as represented by fine particulate 5,5′-dibromoindigo concentrated in the basilar portion of the cell, forming crescentic configurations directed toward the lumen. Intense loci of activity, as well as some perinuclear activity, can be seen occasionally. These are overshadowed by the crescentic distribution (Fig. 3).

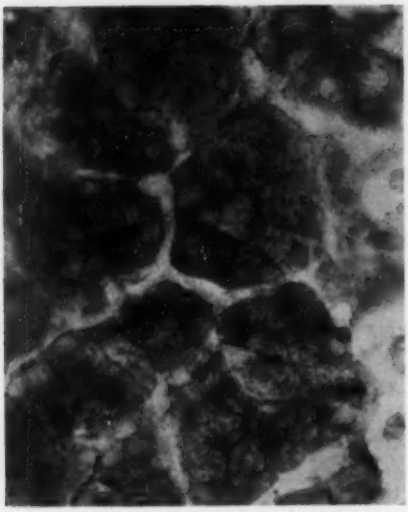


Fig. 4.—Pancreas unfixed but pretreated in M/C at −65 C for 30 minutes. The 5-bromoindoxyl esterase activity is diffuse throughout the cell, especially at the cell borders; × 600.

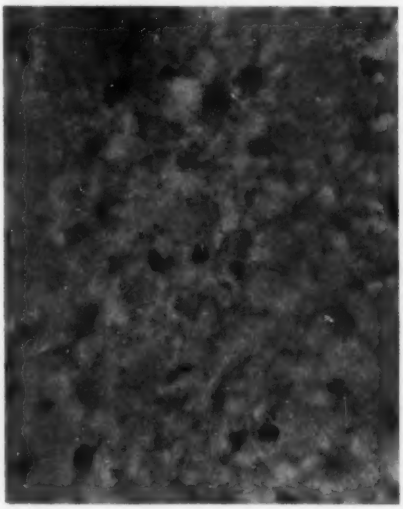
On the other hand, fixed tissue with magnesium ions added to the incubation solution showed the same topographic distribution as above, but the intracellular crescentic distribution was not present. However, there is an intense perinuclear reaction. The basilar portion of the cell also showed intense reaction. The reaction was fine and particulate (Fig. 2). When the tissue sections were fixed in formalin containing calcium ions, the enzyme reaction was concentrated mainly in intense oblong loci running from the midportion of the cell to its luminal border. A faint suggestion of crescents could also be seen. The final reaction product was fine and particulate (Fig. 1).

Pancreas.—If the pancreas is pretreated by M/C and incubated for 15 minutes in the substrate, an extremely fine particulate final reaction product results. The enzymic activity is diffusely distributed throughout the cell. The cell borders are particularly intense. The nuclei are negative. The islands, connective tissue, and vessels are also negative. Some of the ducts show a reaction. A pH of 5 was used. The addition of ions did not alter the intensity or materially alter the intracellular distribution of the enzyme (Fig. 4). In none of these cases could intense loci, such as appeared in the kidney with calcium ions, be seen. Sodium taurocholate enhanced the reaction.

Brain.—The optimal reaction time for the brain was 30 minutes with a pH of 8.1. Intense reaction can be seen in the pia arachnoid and the choroid plexus and to less extent in the basal ganglia. The cortex gives a diffuse reaction. The white matter and vessels are negative. There is some diffusion of the final reaction products to the white matter in practically all instances. Diffusion can be demonstrated in the incubation solution. Thus the brain esterase is extremely soluble. When sodium taurocholate was added, all the enzyme diffused into the incubation solution and the final section was negative. Physostigmine (eserine) does not inhibit the esterase reaction. The addition of ferric, cadmium, and aluminum ions abolished the reaction. Copper ions enhanced the reaction.

Because of this extreme solubility of this indoxyl esterase in the brain, the resulting reaction was somewhat poorly defined. For the most part the cell nuclei were negative.

Fig. 5.—Brain cortex, unfixed but pretreated in F at −65 C for 30 minutes. Benzethonium added to incubation solution. The 5-bromoindoxyl esterase activity is seen in dendrites and perinuclear areas; × 400.



Some perinuclear activity was seen. The intracellular dendritic mesh was positive but not well defined, presumably due to diffusion. The pyramidal cells, astrocytes, oligodendroglia, and microglia all showed the reaction. A section of the cortex can be seen in Figure 5. Here some of the cells can be distinguished. The slide was pretreated with formamide and benzethonium (Phemerol) added to the incubation solution. An intense perinuclear enzyme activity can be seen in most cells of the cortex.

Motor End-Plates.—The motor end-plates can be demonstrated readily in intercostal muscle. They appear in many instances as "organoid" structures having serpiginous appearances. In other instances they appear as "foot plates" on the surface of the muscle. Occasionally one can trace the terminal end of the intercostal nerves as they react with the indoxyl substrate. They react very rapidly with 5-bromoindoxyl acetate to form a fine precipitate of the resultant final reaction product (Fig. 6).

With most additive ions diffusion of the final reaction product is prominent, and so portions or whole muscle fibers give the reaction. The addition of ferric, ferrous, cadmium, and aluminum ions gives no reaction. The optimum pH is 7.2, and the optimum incubation time is 10 minutes. The Standard Solution I was the most favorable. Eserine abolishes the reaction completely.

Fig. 6.—Intercostal muscle, showing motor end-plate. Tissue is unfixed. Zinc acetate is added to incubation solution. Intense 5-bromoindoxyl esterase activity is seen, forming a serpiginous appearance; × 1,100.

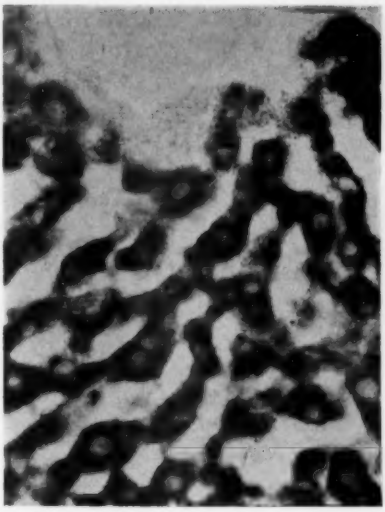


Fig. 7.—Liver is unfixed and unpretreated. The 5-bromoindoxyl esterase activity is uniform through the lobules and only in the cytoplasm of hepatic cells; × 400.

Liver.—The optimum incubation time for the liver was 15 minutes, and the pH was 5. Standard Solution II gave the best results. There was a uniform esterase reaction throughout the whole hepatic lobule, involving the cytoplasm. The nuclei, Kupffer cells, bile ducts, and vessels were negative (Fig. 7). No effect could be seen with additive ions.

Bone Marrow and Peripheral Blood.—Many cells of the bone marrow showed intense esterase reaction. Most of these cells were mononuclear and were difficult to identify. The eosinophilic myelocytes were negative as well as the megakaryocytes. This is in contradistinction to the work of Zajicek,[10] who found that megakaryocytes of rats gave a positive reaction with the butyralthiocholine techniques of Koelle and Friedenwald. The peripheral blood showed intense reaction in the mononuclear cells. A

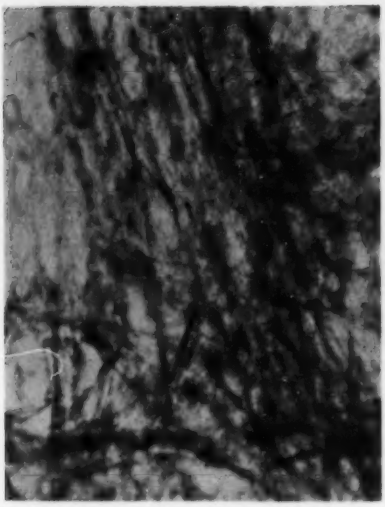


Fig. 8.—Section of thrombus, unfixed and unpretreated. Fibrin strands show intense 5-bromoindoxyl esterase activity; × 400.

much fainter reaction could be seen in the polymorphonuclear cells. An incidental finding of interest occurred in the examination of the formation of thrombi in the peripheral blood. Just prior to thrombus formation a fine precipitate of 5,5′-dibromoindigo appeared at various loci. This quickly became intense and was present in thrombin fibers as they were laid down. All thrombi in both rat and human showed intense esterase reaction (Fig. 8). Incubation Solution IV was used.

Comment

The substituted indoxyl substrate seems an ideal one for the demonstration of the final reaction product; nevertheless, several problems remain for the more decisive identification of esterases in tissue. Also, because of lipids which may accumulate in pathologic material, it would seem desirable to remove these without denaturation of the enzyme.

We previously found that lipids could be removed without alteration of the enzyme, succinic dehydrogenase, by pretreating the tissue section at −65 C for 50 minutes.[7] This has also been found to be true with nonspecific esterase, lipase, and cholinesterase. The character of the solvent mixture was not a factor in the preservation of the enzyme.

Important differences could be demonstrated in the brain and motor end-plate esterase, even though previously assumed that the enzyme was the same. These differences can be categorized as follows. Eserine abolished esterase completely in the motor end-plates but not in the brain. The incubation time was 10 minutes for the motor end-plates and 30 minutes for the brain, and the optimum pH requirements were 7.0 and 8.1, respectively. The addition of ferric, cadmium, and aluminum ions inhibited both these esterases, whereas ferrous ions inhibited only the motor end-plates. The effect of formalin fixation showed that the "half-life" of the enzyme was two hours for the motor end-plates and four hours for the brain. The brain esterase showed marked solubility, which differed somewhat from that of the motor end-plates. When zinc ions were added, no effect could be seen in the motor end-plates, while the brain esterase was practically completely inhibited. The subsequent addition of glycine restored the enzyme activity of the brain, and a strong histochemical reaction became apparent. The zinc ions form a complex with the imidazole groups, which blocks the esteritic site of enzyme action. The zinc can presumably be released by the addition of glycine. No explanation can be given for the failure of this to occur in the motor end-plates.

The 5-bromoindoxyl acetate was also used by Pepler and Pearse [11] to demonstrate rat-brain esterases, particularly those of the basal ganglia. They distinguished six types of esterases in brain tissue. Under Type I they describe "nonspecific esterase" of most neurons of the cerebral cortex. As in our findings, this was not inhibited by eserine. However, their results differ from ours in that the optimum pH was 6.2.

The fact that imidazole, as well as histamine, gives positive reactions nonenzymatically implicates the imidazole group. Bruice and Schmir,[9] using phenyl acetate as a substrate, found that imidazole catalyzed the

hydrolysis of this and other related esters. Wilson and Bergmann [12] suggested by measurement of the ionic constants that imidazole could be a possible functional group in cholinesterase. Doherty and Vaslow [13] suggested that in the formation of a chymotrypsin substrate complex the binding might be ascribed to the ionized imidazole groups. Hammond and Gutfreund [14] showed possible evidence that the imidazole group of histidine is the catalytic site of chymotrypsin. We found that chymotrypsin also splits 5-bromoindoxyl acetate. This would indicate that 5-bromoindoxyl acetate is not as specific as formerly thought and that when it is used other modes of separation of the enzyme must be employed.

We found that free imidazole was inhibited 100% by the addition of zinc ions, whereas histamine is inhibited about 75%. Recently Clouet et al.[15] found free histamine and histidine in the rat brain of 26.1μg. per gram and 4.3μg. per gram of fresh brain, respectively. This may then suggest that these free groups in the brain containing imidazole catalyze the hydrolysis of 5-bromoindoxyl acetate, thus giving a pseudoreaction for esterase which may explain the solubility and diffusion in the brain.

The use of $CaCl_2$ in the fixation of the kidney gave results similar to those reported by Holt [8] as discrete loci of enzyme activity. These were also described in pancreas and liver by Holt [8] and by Novikoff and Holt.[16] The diffuseness of the resultant final precipitation product was interpreted as intracellular diffusion. We were unable to demonstrate this in liver and pancreas by the addition of various ions.

The finding of negative reactions in the megakaryocytes was of interest, since Zajicek [10] demonstrated esterase in these cells by Koelle's method. The incidental finding of esterase activity in thrombus formation is of interest, since Seegers and Landaburu [17] have found recently a thrombin esterase during the conversion of fibrinogen to fibrin. Mast cells in lymph node and thymus were intensely reactive, and all the polymorphonuclear cells in the peripheral blood except the eosinophils were positive. It is possible that this may be related to their histamine content and not to their esterase.

Summary

Although 5-bromoindoxyl acetate is an ideal substrate in regard to its final reaction product, it is nonspecific for esterase. It is hydrolyzed by chymotrypsin, lipase, nonspecific esterase, and cholinesterase, as well as by free substances containing imidazole groups. The enzyme is not altered by pretreatment at −65 C with various solvents which extract lipids from tissue. Intracellular shifting of the final reaction products can be demonstrated in the kidney as an effect of the addition of various ions. Important differences can be shown between esterase reaction in brain and in motor endplates.

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Intestinal Pneumatosis

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Intestinal pneumatosis is an abnormal collection of gas in the walls of the intestine or in its adjacent structures, e. g., the mesentery, ligaments, or lymph nodes. It has been reported under various titles: pneumatosis cystoides intestinalis, pneumatosis intestinales, gas cysts of the intestine, abdominal gas cysts, and intestinal emphysema.

Long before the report of the human disease, intestinal pneumatosis had been noted in animals. Mayer(1825) coined the term "pneumatosis cystoides intestinorum" after observing these cysts in the intestine of hogs. Bang [2] (1876) is credited with the first adequate description of the disease in humans when he noted gas cysts in the intestine of a woman who died of a volvulus. Finney [7] (1908), reporting the first case in the American literature, observed a mass of cysts in the ileum of a patient with pyloric obstruction caused by carcinoma of the stomach. Although a recognized entity since 1876, the mechanism of this disease has not been clearly explained.

In the files of the Indiana University Medical Center, this is the only case of intestinal pneumatosis among nearly 9,000 autopsies. Although uncommon, these cysts are reported with increasing frequency. Koss [12] (1952) reviewed 255 cases in the world literature and analyzed 213 which he considered to be proven intestinal pneumatosis.

Report of a Case

A white woman, age 45, was admitted to the Indiana University Medical Center, complaining of bilateral hip pain aggravated by exercise, coldness, and numbness of both lower extremities of eight days' duration.

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Rheumatic fever had occurred at age 7 years. Signs and symptoms of rheumatic heart disease were present at age 27 and prompted the use of digitalis. Two years before admission, mitral valvulotomy was suggested because of advanced mitral stenosis but the patient refused. An appendectomy was performed 25 years previously.

Physical examination revealed a blood pressure of 140/60 mm. Hg. The heart rate was 68 per minute and irregular; the temperature, 98.0 F. The left border of the heart extended to the anterior axillary line in the sixth intercostal space. An early diastolic murmur at the apex suggested mitral stenosis. Both legs were mottled and cool, and sensation to pain was diminished in the left leg. The pulses of the femoral, popliteal, posterior tibial, and dorsalis pedis arteries were not palpable bilaterally. The remainder of the physical examination was not contributory. The clinical diagnosis was (1) rheumatic heart disease with mitral stenosis and insufficiency, left ventricular hypertrophy and dilatation, and auricular fibrillation, functional, Grade II; (2) "saddle embolus" at the bifurcation of the aorta.

On the 25th hospital day a mitral valvulotomy was performed. Postoperatively, there was a precipitous peripheral vascular collapse. The systolic blood pressure fell to 80 mm. Hg. The abdomen was rigid, distended, and apparently tender. Several watery stools were passed. Despite intensive supportive therapy, the patient failed to respond and died on the second postoperative day.

At postmortem examination the heart weighed 475 gm. The mitral and aortic valves were thickened, fused, and stenotic. On the surface of the spleen and kidney irregular bands of fibrous tissue, suggestive of healed infarcts, distorted the normal shape of the organs. At the bifurcation of the aorta, a firm, dark red, friable thrombus filled the lumen and was impacted into the external iliac arteries. The thrombus extended 7 cm. above the bifurcation of the aorta and occluded the orifice of the inferior mesenteric artery. The superior mesenteric and renal arteries were patent. The stomach was dilated and contained approximately 600 ml. of red-brown fluid resembling partially digested blood. The mucosa was intact but congested and covered with petechiae and ecchymoses.

The mucosa of the small and large intestine was congested and had lost some of the plical pattern, suggesting necrosis and autolysis. The lumen was

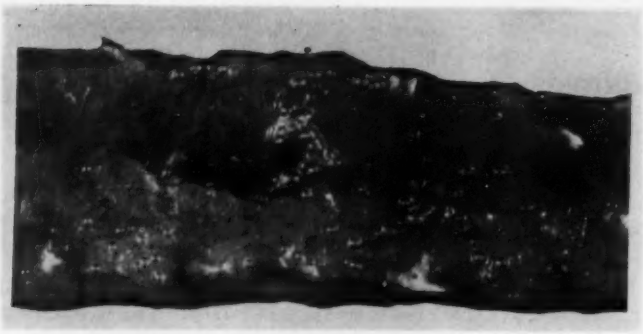

Fig. 1. — Collection of cysts observed on the mucosal surface of the intestine.

filled with dark red fluid. Discrete ulcerations were not found. Petechiae and ecchymoses were prominent in the small intestine but were also noted in the colon. Clusters of glistening, translucent cysts, varying in size from 1 mm. to 10 mm. covered the mucosal surface of the small and large intestine, including the duodenum. On puncture, the cysts collapsed and no fluid was released. On palpation, there was a sensation of crepitus similar to that experienced in palpating a normal lung. The cysts were most numerous in the ileum, cecum, and ascending colon, but clusters were also found in the duodenum and jejunum and in the transverse, descending, and sigmoid colon. In the duodenum the cysts did not differ in appearance from those in other portions of the bowel.

Microscopic examination of the liver, pancreas, and adrenal glands revealed no significant abnormalities. In the lungs there was evidence of chronic passive congestion. Moderate fibrosis was noted in the adventitia of small blood vessels of the myocardium. Aschoff bodies were not present. Wedge-shaped scars extending from the cortex to the medulla of the kidneys were considered to be healed infarcts. In the intima of the abdominal aorta, there was moderate arteriosclerosis. The thrombus found in the lumen of the aorta was not attached to the wall.

Multiple sections taken throughout the intestine varied little in microscopic appearance. The overlying glandular epithelium was partially necrotic and autolyzed. Islands of normal-appearing glands were separated by cellular debris. The cysts, found exclusively in the submucosa, were empty. Some of the cysts were lined by fibrous tissue. Others were lined in part by flattened or cuboidal cells. The nuclei of these cells were dark; the cytoplasm, eosinophilic. Giant cells were not noted in any of the microscopic sections. Loose connective tissue separating the cysts was well vascularized and contained occasional mono-

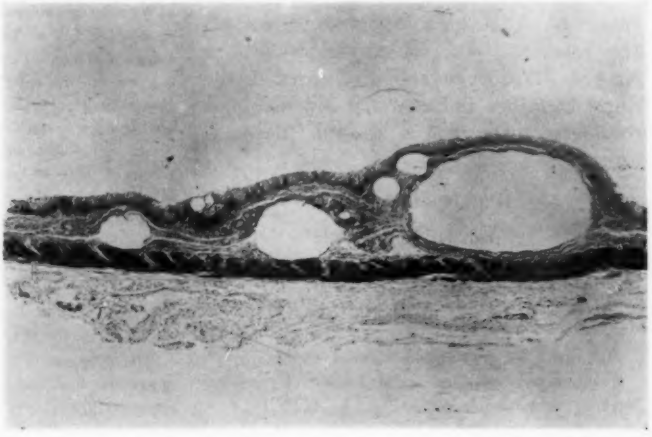

Fig. 2.—Typical group of cystic spaces in the submucosa of the intestine.

nuclear cells, predominantly histiocytes. Bacteria were not demonstrated histologically in the cyst wall. The histologic findings in other tissues and organs were not significant. The mucosa of the stomach was intact.

Comment

Intestinal pneumatosis is usually associated with some other lesion of the gastrointestinal tract. In approximately 50% of cases in adults, there is an ulcer of the stomach or duodenum, usually with pyloric stenosis.[12,15] Less frequently, there are other lesions which cause a loss of continuity or integrity of the intestinal mucosa: acute and chronic colitis, nonspecific enteritis, and acute peritonitis.[3,12] Stiennon[22] reports a case of a 4-week-old infant in whom submucosal gas cysts were associated with infarcts of the small bowel and chronic catarrhal colitis. In infants, the disease is usually associated with an acute enterocolitis[9,14,16] although epithelial ulceration is not an invariable concomitant of intestinal pneumatosis. When no associated lesion of the gastrointestinal tract is found, as was the case in 15% of those reported by Koss,[12] the disease is considered to be primary intestinal pneumatosis.

The cysts are segmentally distributed. A predilection for the terminal ileum and cecum is commonly cited, but cysts have been reported in all segments of the gastrointestinal tract. However, cysts located in the stomach and duodenum are rare. Plenge cites a case reported by Kolli[11] in which cysts were described in the duodenum. Involvement of the entire wall of the intestine is also a rarity. Occasionally, "skip areas" are noted in which noncontinuous segments of the intestine are covered with cysts.

The location of the cysts in the intestinal wall is variable. A perusal of the literature reveals that in adults, in association with other lesions of the gastrointestinal tract, e. g., peptic ulcer, the cysts are more frequent in the subserosa, usually on the antimesenteric border. Submucosal cysts have been reported, however, in association with other gastrointestinal lesions.[23] When no intestinal disease coexists, the submucosal location is commoner but cysts have also been found in the serosa.[1,20,21] Cysts may be found simultaneously in both submucosal and serosal layers.[1,13,23] Less frequent extraintestinal locations are the gastrohepatic ligament, falciform ligament, mesentery, mesocolon, omentum, and mesenteric lymph nodes. In infants, the cysts tend to occur in association with an entercolitis and the submucosal site predominates.[14,16]

The size of the cysts varies from 1 mm. to 5 cm., and they occur singly or in clusters suggesting soap bubbles or clusters of grapes. The cysts collapse on puncture and contain an odorless gas, although cysts containing small amounts of fluid have been reported. Microscopically the cysts are partially or completely lined by a layer of cells, which are usually flattened or cuboidal. Columnar epithelium is rarely seen. Giant cells, a prominent feature of the disease, are frequently seen in the wall of the cyst or actually lining the cavity. Between the cysts is dense fibrous tissue, with a minimal inflammatory response consisting primarily of mononuclear cells with occasional eosinophilic and neutrophilic polymorphonuclear leukocytes. In children, giant cells are not frequently observed.

Because of the prominent lining of flattened cells, it is assumed that the gas is present in vascular channels, probably lymphatics, although this has not been proven conclusively. In some reported cases, the cavities are lined only by compressed fibrous tissue. It is postulated that in cysts of recent origin the lining is absent, and in more chronic cases, endothelial cells, giant cells, and fibrosis become prominent. Koss[12] excludes cases in which cysts are without an endothelial lining and suggests that in some of these, developing shortly before death, intestinal gas penetrates into the wall of the intestine and not into the lymphatics selectively. Hence, an endothelial lining is absent.

Frequent reference is made to the experimental work of Wright,[25] who injected oxygen, nitrogen, and carbon dioxide subcutaneously and produced spaces lined by flat mesothelial-like cells. There was also an accumulation of epithelial cells and giant cells of the foreign-body type. This experimental work suggests that gas alone, after entry into the bowel wall, may induce the changes seen microscopically in intestinal pneumatosis.

Most of the cases of intestinal pneumatosis are asymptomatic and are noted at autopsy or laparotomy for another disease. However, recent reports in the literature indicate that these abnormal accumulations of gas may be related to vague and previously undiagnosed complaints of the abdomen. Submucosal cysts may protrude into the lumen and narrow and intermittently obstruct it.[8,12] These cysts apparently can be the cause of rectal bleeding, diarrhea, and lower abdominal pain.[1,6,13,17] Cysts located in the sigmoid colon have been visualized through the sigmoidiscope[17] and in some instances have been mistaken for adenomatous polyps.[1,19] In reviewing the pediatric literature, Paris[16] could find no case in which intestinal obstruction was caused principally by submucosal cysts, but diarrhea was a prominent symptom. Subserosal cysts may exert extrinsic pressure and narrow the lumen of the bowel. A volvulus may result from a mass of cysts.[2,12] Rupture of the subserosal cysts cause pneumoperitoneum.[5,20]

The cause of intestinal pneumatosis is not known. The neoplastic and infectious theories are only of historical interest. Finney,[7] in association with Prof. William Henry Welch, was of the opinion that the cells lining the cysts were of neoplastic origin and had the capacity of producing gas. The clinical course of these patients, the histopathologic findings, and the observation that the cysts may spontaneously disappear lend little support to this theory.

There have been no conclusive bacteriologic studies to substantiate the theory that bacterial action produced the gas. No organism has been cultured consistently from the tissues or cysts. The microscopic findings in cases of intestinal pneumatosis do not suggest a bacterial infection. In a case report of fatal enteritis caused by Clostridium welchii, a bacterium known to produce gas, Kellert and Meeler[10] noted vesicular spaces without lining cells. A submucosal and mucosal cellular infiltration consisting of mainly neutrophilic leukocytes was present, together with clusters of Gram-positive bacilli.

The experimental work of Biester, Eveleth, and Yamashiro[4] is frequently mentioned to substantiate the theory that a nutritional deficiency is a factor in the formation of the cysts. These workers observed intestinal cysts in 16 of 22 pigs fed a diet of polished rice. They were unable to identify the substance the absence of which was responsible for the disease. The association of intestinal pneumatosis with other digestive disturbances suggests that a nutritional factor may play some role.

The mechanical theory is most widely accepted. It is postulated that gross and microscopic defects of the intestinal mucosa allow the entrance of gas from the intestinal lumen. With hyperperistalsis or an increase in intraluminal pressure, gas is forced through the defect into the tissue spaces by way of lymphatic channels. Tung and Ngai,[24] while operating on a patient with a peptic ulcer, observed the presence of cysts in a segment of the small intestine. Vomiting occurred during the laparotomy, and they noticed an increase in size of the cysts although the intestine itself did not become distended. This demonstration of an apparent direct association between intestinal cysts and a stenosing lesion of the duodenum has never been demonstrated by other workers.

Although the mechanical theory is a plausible explanation of cysts seen adjacent to diseased loop of bowel, it does not explain the fact that cysts are often some distance

from the mucosal ulceration, e. g., intestinal pneumatosis with pyloric obstruction. Stiennon [22] suggests that there is retroperitoneal dissection of gas from the site of ulceration in the stomach or duodenum to the mesenteric root. From the mesenteric root, gas dissects along the subserosal layers or follows the blood vessels and enters the submucosa. However, the rarity of mesenteric cysts in association with cysts in the bowel wall is not easily explained by this theory.

The case reported here presents certain interesting findings. As mentioned previously, cysts involving the entire intestine including the duodenum are exceedingly rare. In our case, discrete glistening cysts were noted on the mucosal surface of all divisions of the bowel. The cysts were partially lined by flattened and low cuboidal cells, which suggests that these cysts may be dilated lymphatics. In reviewing the history, there was some clinical evidence of intestinal irritation, although this was secondary to the more important problem of shock. It has been shown that the mucous membrane is the first part of the intestine to be affected by a deficient blood supply. A discontinuity of the bowel mucosa is suggested by the numerous petechiae and ecchymosis of the mucosa and by the presence of unclotted blood in the lumen. We postulate that these numerous microscopic tears in the mucosa allowed gas to dissect into the wall, probably by way of the lymphatics. Irritation, probably from blood, led to hyperperistalsis and diarrhea. The clinical course and histopathologic findings would indicate that the interval following occlusion of the mesenteric artery was insufficient for the development of extensive necrosis.

Summary

A case of intestinal pneumatosis involving the small and large intestine is reported. Involvement of the duodenum is a rare finding. The history, distribution, and theories of pathogenesis are briefly considered.

Photomicrographs were taken by Mr. Paris Johnson and Mr. Joseph Demma.

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Fatal Cases of Acute Sicklemia

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Acute sicklemia in patients with sickle-cells trait raises problems very different from manifest clinical sickle-cell anemia. Those with sickle-cell trait present no anemia or symptoms except during time of crisis. Such an acute crisis may take the form of hematuria [1-5] or multiple infarcts, which may occur in the spleen only during high-altitude flight [6-10] or in multiple organs.[11-12] These complications are very rare and are not generally recognized. Recent experience suggests that they may be commoner than is suspected.

Report of Cases

CASE 1.—The patient was a 56-year-old Negro woman, admitted to St. Elizabeth Hospital on Dec. 6, 1955, with marked disorientation. She previously had a diagnosis of toxic nodular thyroid disease, followed by digitalization and subtotal thyroidectomy. Physical examination revealed a blood pressure of 170/100 mm. Hg, peripheral arteriosclerosis, recent thyroidectomy scar, and old depressed scar on the forearm. A diagnosis of manic depressive psychosis, manic phase, was made. Six days later (Dec. 12), the patient was found with unobtainable blood pressure and pulse; respiration was rapid and shallow, with extreme cyanosis of hands and feet. Physical examination revealed a well-developed, undernourished Negro woman with markedly cyanotic hands and feet. Positive physical findings included scattered rales at both bases, a Grade 2 apical systolic murmur over the entire precordium, unobtainable blood pressure, very weak but regular pulse of 76 per minute, and a subnormal temperature of 94 F. The abdomen was flat, rigid, and silent, but no palpable organ was found. The liver appeared of normal size by palpation and percussion, but the right upper quadrant was very firm and tender to palpation. The patient repeatedly stated, "My stomach hurts," and, "I am thirsty," although she was very much out of contact. After methoxamine (Vasoxyl) and fluid were administered, the blood pressure promptly rose to 160/100 mm. Hg and the temperature also rose to a normal level. The next day the patient was active and the blood pressure remained at 120/80 mm. Hg but right upper quadrant and right flank tenderness persisted. Two days after admission the temperature rose to 104 F, with no chill, and the patient passed a large tarry stool. The blood pressure again fell, and intravenous levarterenol ([*l*-norepinephrine] Levophed) and cortisone were instituted. She then became comatose, with both eyes deviated to the left but no other neurological signs. The blood pressure was 110/65 mm. Hg; pulse, 78, and respirations, 24 per minute at this time, and there was livid cyanosis of the distal ends of the digits of hands and feet. The blood pressure fell each time when levarterenol was discontinued. The electrocardiogram showed no significant findings, and the patient received continuous intravenous tetracycline (Achromycin), digitoxin, cortisone, and levarterenol and also 500 cc. of whole blood twice. Although generally alert, she showed progressive discoloration of digits until Dec. 19, 1955, when she finally became lethargic. At the same time, the blood pressure remained at 80 to 90 mm. Hg systolic and 60 mm. Hg diastolic. The temperature fluctuated from 101 to 104 F until the time of death, on Dec. 22, 1955, ten days after the onset of unobtainable blood pressure and pulse. The complete blood cell count made during admission, revealed 3,500,000 RBC and 14,800 WBC per cubic millimeter, with 92% neutrophils and 8% lymphocytes. Hemoglobin was 10.4 gm., and hematocrit was 32.5%. Sickling was not noticed on the blood smear examined by the technician. The hematocrit dropped to 28% on Dec. 13, 1955, but it rose to 32% after a transfusion. Urine showed 4+ proteinuria on one of three examinations. One + glycosuria and 4-5 WBC per high-power field were also noted. The urine culture revealed Escherichia Coli on Dec. 16, 1955. The admission N. P. N. was 152 mg.; fasting blood sugar, 263 mg.; CO_2, 18.5 mEq. per liter; total protein, 6.3 gm., with 4.7 gm. % albumin. The N. P. N. remained high, 121-150 mg. %, except for one examination, on Dec. 17, which was only 70. Fasting blood sugar three days after admission was 484 mg. %. The CO_2 varied from 10.3 to 25 mEq. per liter; chlorides, from 116.5 to 126.3 mEq.; sodium, from 133 to 134 mEq., and potassium from 3.4 to

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4.5 mEq. Liver-function studies made three days after admission revealed serum bilirubin of 1.78 mg. %; thymol turbidity, 2 units; serum cholesterol, 227 mg. %, and cephalin flocculation, 3+ in 48 hours. Blood culture, on Dec. 13, 1955, revealed α-Streptococcus and Aerobacter aerogenes, considered as contaminations.

X-ray films of the chest and abdomen were reported as not significant.

At autopsy, there were the following significant findings: There was slight malnutrition, and the skin had many superficial, well-defined, and reddish-black areas in the hips, sacrum, and shoulders. A 6 cm. decubital ulcer was present over the sacrum. The right lower extremities had pitting edema and a large superficial ulcer, involving the lower two-thirds of the leg. The distal phalanges of fingers and toes showed dry, blackish gangrene. A 10 cm. and healed collar-shaped surgical scar was present on the anterior lower neck. The heart weighed 300 gm., and all the chambers were slightly dilated and very flabby. Scattered petechial hemorrhages, not larger than 3 mm., were seen on the mitral leaflets and aortic cusps. The aorta and large arteries showed slight arteriosclerosis but no thrombus. The right and left lungs each weighed 400 gm. and showed a small amount of reddish fluid on the cut surface but no pneumonic or infarcted areas. The spleen weighed 240 gm. and had a well-defined, wedge-shaped, partly fresh hemorrhagic, partly yellowish infarct, 2.5 cm. in diameter (Fig. 1), with no demonstrable thrombotic or embolic occlusion of the splenic artery and vein. The liver weighed 1,500 gm. and had large areas of subcapsular hemorrhage, more over the anterior and superior surfaces of the right lobe. The sectioned surface revealed several ill-defined softened and reddish areas (Fig. 2), averaging

Fig. 1 (Case 1).—Spleen, showing large, partly hemorrhagic, and partly anemic infarct.

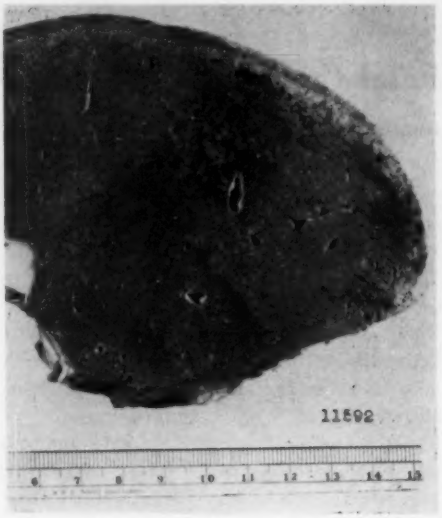

Fig. 2 (Case 1).—Liver, showing ill-defined, softened, and reddish areas in the right lobe.

3×4 cm. in diameter but no distortion of the lobular pattern. The mucosa of the stomach presented several petechial hemorrhages. The right and left kidney each weighed 180 gm., with several minute hemorrhages in the medulla and the epithelial surface of the calyces and pelvis. The ovaries had large areas of hemorrhage on the sectioned surface. The bone marrow was moist and reddish, and the right and left lobes of the thyroid each measured 3.5×2.5×1.5 cm. At the lower pole of the left lobe there were two circumscribed nodules, the largest one being 1 cm. in diameter. The capsule was slightly thickened and adhered to the adjacent muscles. The brain weighed 1,210 gm., and on coronal section a small cystic infarct was found to be present in both the right and the left globus pallidus, each measuring 3 mm. in diameter.

Microscopic studies revealed the *spleen* had coagulation necrosis, with focal areas of neutrophilic infiltration and marked dilatation and congestion of all the sinusoids. There was pronounced sickling phenomenon in areas adjacent to or in the necrotic areas. The section of the *liver* showed moderate increase of portal spaces with connective tissue, which was widely separated with fresh hemorrhage, between the lobules. Many of these red blood cells were seen to be definitely sickled-shaped. The liver cords of the remaining part of the lobules

Fig. 3 (Case 1).—Liver (low magnification), showing alteration of lobules, portal spaces markedly increased by dilated vessels and fresh hemorrhage; × 100.

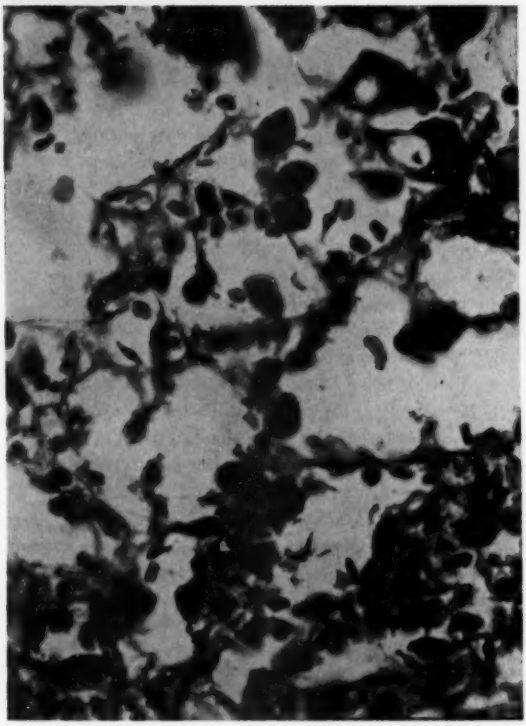

Fig. 4 (Case 1).—Liver (high magnification), showing the connective tissue in the portal space widely separated by dilated vessels and fresh hemorrhage with sickled red blood cells; × 470.

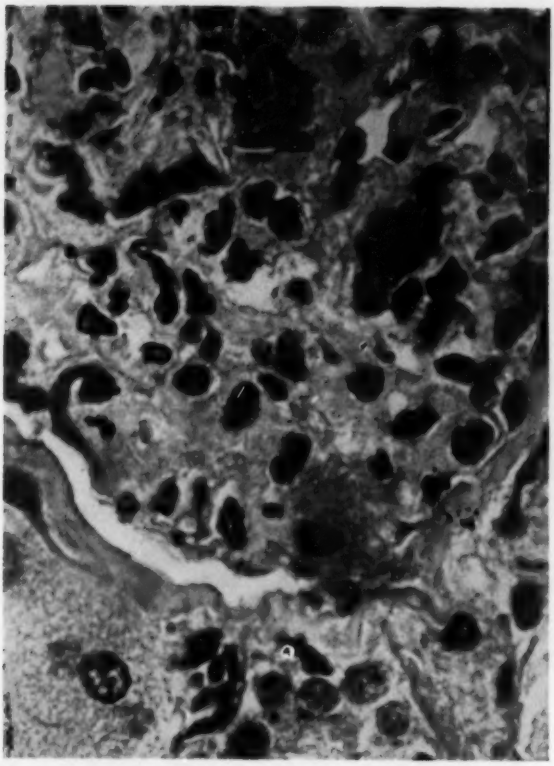

Fig. 5 (Case 1).—Kidney, showing capillary thrombosis with sickled red blood cells. Oil immersion; × 970.

were widely separated and moderately disorganized by the markedly distended sinusoids, as well as the central veins (Figs. 3 and 4). There were moderate fatty metamorphosis and hemosiderin pigments in the Kupffer cells. Many renal glomeruli had thrombosed capillary tuffs from the agglutination of sickle-shaped red blood cells. Many of the small veins were engorged and dilated, filled with similar sickle-shaped red blood cells (Fig. 5). The bone marrow was rather fatty and hypoplastic, with all the cellular elements present but no hyperplasia of the erythrocytic series. The section of the globus pallidus revealed demyelinization, gitter cells, and packed sickle-shaped red blood cells in many of the blood vessels.

Case 2.—The second case was an 86-year-old Negro man, admitted to St. Elizabeth Hospital in 1942, with a diagnosis of senile psychosis. A hemogram done on March 23, 1956, showed hemoglobin, 10 gm. %; RBC, 4,250,000 per cubic millimeter, and WBC, 7,800 per cubic millimeter, with a not unusual differential count. The past history revealed a long-standing hypertensive cardiovascular disease compensated on digitalis and diuretic therapy. He also had several episodes of pneumonia during his 10-years stay in the hospital. He was transferred to the medical and surgical service on Jan. 17, 1957, with a history of lethargy and slight elevation of temperature. The present illness started on Jan. 9, 1957, when the patient had projectile vomiting, and physical examination during admission revealed a temperature of 100 F; blood pressure, 150/100, and irregular pulse. The positive findings included bilateral early cataracts, coarse and moist rales and expiratory wheezing over the left base, and enlargement of the heart to the left. Frequent extrasystoles were present. Hemogram during admission revealed hemoglobin 12.8 gm. %; hematocrit, 42 vol. %, and WBC, 10,400 per cubic millimeter, with a not unusual differential count also. The impression on admission was pneumonitis of the left lower lobe and questionable digitalis intoxication, with trigeminy premature ventricular

contractions. Digitalis was discontinued. On Feb. 27, 1957, he, for the first time, complained of pain in the epigastrium. The next day he was about to be transferred back to psychiatric service, but he was found to be acutely ill, with unobtainable peripheral pulse and blood pressure. Shortly afterward, he vomited 1,000 cc. of gastric contents, and the blood pressure and pulse returned. On examination, the abdomen was found to be soft, and the lungs were clear to auscultation and percussion. Two hours, later, the patient was again in shock, with a very tense and tender abdomen; surgical consultants did not agree that this was acute surgical abdomen, and the patient was treated with intravenous fluid, levarterenol, and oxygen, with no significant effect. The patient died at 11:05 p. m. on the same day, Feb. 28, 1957.

The autopsy was performed five days after death and revealed a senile, 86-year-old, well-developed and fairly well-nourished Negro man, weighing approximately 140 lb. The important findings included herniation of the cardiac end of the stomach through the hiatus hernia into the left pleural cavity, anteriorly and to the left of the descending aorta, and a circular fibrous weakening of the right dome of the diaphragm, 7 cm. in diameter, covering the slightly herniated superior portion of the right lobe of liver. The *heart* weighed 500 gm., with dilatation of all the chambers. The myocardium of the left ventricle measured 20 mm., and the mitral and aortic valves showed yellowish fibrous thickening along the ring. The aorta showed very marked arteriosclerosis, more marked in the abdominal portion. The *right lung* weighed 720 gm. The upper and middle lobes showed moderate edema, and the lower lobe revealed many irregular, slightly elevated, yellowish-gray and firm areas, in contrast with the surrounding dark reddish lung parenchyma. The *left lung* weighed only 440 gm. and showed marked fibrous adhesion covering the entire pleural surface. The sectioned surfaces of the left lung also showed edema and congestion. No infarct was recognized grossly. The *spleen* weighed 320 gm. The capsule, as well as the sectioned surface, showed many well-defined, large yellowish-to-dark-purplish-red infarcts. The yellowish ones appeared to be firm, and the dark purplish red ones, to be hemorrhagic and liquified. The largest infarct, which was liquified and hemorrhagic, measured 5×3 cm.

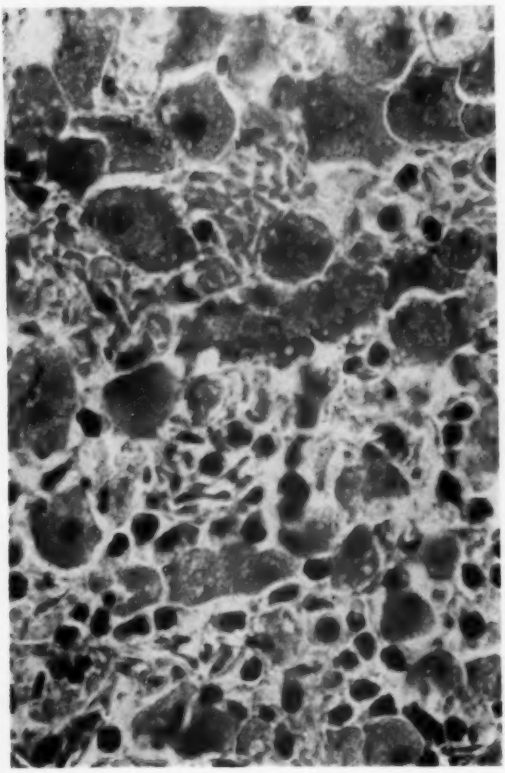

Fig. 6 (Case 2).—Liver (high magnification), showing dilatation of sinusoids with sickled red blood cells and early necrosis of liver cells with inflammatory reaction; × 470.

The splenic vein and splenic artery showed no thrombosis, but the latter was markedly tortuous and arteriosclerotic. The *liver* weighed 1,400 gm. The anterior surface of the right lobe had a hemorrhagic area, 7×5 cm. in diameter, and over the inferior surface there were a number of small, well-defined, and yellowish areas, which on sectioning were well demarcated and slightly firm, the largest one measuring 2.5×1.5 cm. in diameter. In addition, there were many ill-defined large and yellowish-brown areas involving the whole liver, in contrast to the remaining dark-reddish-brown liver parenchyma. The lobules, however, were very uniform and unaltered. There was no thrombosis of the portal vein, hepatic vein, or hepatic arteries. The *kidneys* each weighed 120 gm. Both showed coarse granularity and multiple scars, the largest one measuring 1.2×1 cm. The left kidney, in addition, also showed three acute infarcts—the largest one, measuring 2.5×1.5 cm., involving the entire cortex as well as the medulla. The stomach and intestinal tract were not remarkable, with no hemorrhage or infarct. The brain weighed 1,120 gm., and the dissection after fixation showed marked arteriosclerosis of the cerebral arteries and slight ventricular dilation. The right caudate nucleus, which was smaller than the left one, showed definite scarring. There was also a decrease in the volume of the putamen and the globus pallidus and the internal capsule in the same right side, extending into the mamillary bodies.

Microscopically, the lungs revealed bronchopneumonia and fresh hemorrhagic infarct with no inflammatory reaction, but many of the small and large pulmonary vessels were dilated and congested with sickle-shaped red blood cells. The *spleen* showed extensive fresh infarction, and the sinusoids, as well as the small and large splenic veins, were filled and distended with sickle-shaped red blood cells. The liver revealed coagulation necrosis of different ages. All the sinusoids and small and large veins, including the portal and hepatic veins, were distended and filled with sickle-shaped red blood cells. The sickling phenomenon was much more conspicuous in the areas which were adjacent to the infarction (Fig. 6). The *kidneys* showed very prominent capillary thrombosis of the glomeruli by the sickle-shaped red blood cells (Fig. 7). There was no evidence of erythrocytic hyperplasia in the bone marrow.

Comment

It is unfortunate that the diagnosis in these two cases of acute sicklemia with

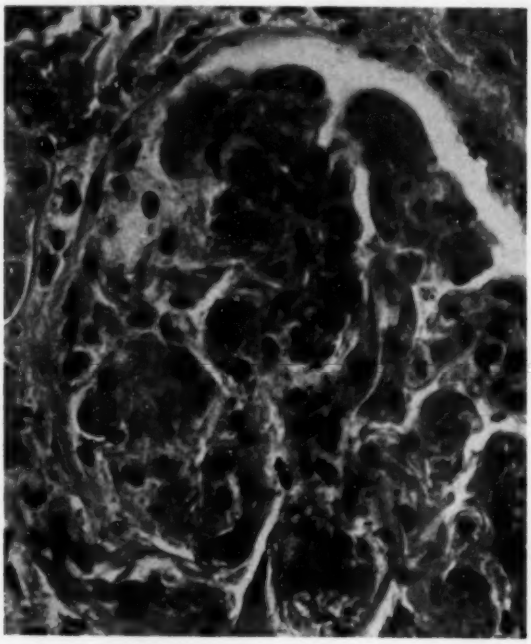

Fig. 7 (Case 2).—Kidney (high magnification), showing capillary thrombosis and congestion of interlobular veins with sickled red blood cells; ×470.

infarcts of multiple organs was not suspected clinically in order that sickle-cell preparation and especially immunological studies could have been done to determine the components of the patients' hemoglobin. It has been reported that sickle-cell hemoglobin C disease is particularly prone to sickling in low oxygen tension.[13] Sickle-cell hemoglobin C disease, like sickle-cell anemia, is a hemolytic disease but could be compensated by bone marrow overproduction without evidence of anemia. Both of these cases had mild hypochromic and microcytic anemia, which is a not uncommon finding in the institutionalized patients of the Negro race. There was also no fibrosis or siderosis of the spleen or erythrocytic hyperplasia of the bone marrow to indicate the existence of sickle-cell anemia. The routine peripheral blood smear examination also shows no sickling of red blood cells. All these findings would probably eliminate the possibility of sickle-cell anemia, but it is impossible to state whether the patient had sickle-cell trait or sickle-cell hemoglobin C disease.

From the history and clinical course it is very apparent that the first patient had developed acute sicklemia during the time of admission, with pain and tenderness in the upper abdomen which is most likely due to infarct of the spleen 10 days before her death. This assumption is further supported by the histological findings of neutrophilic infiltration in the infarcted areas and the appearance of free iron in the Kupffer's cells of the liver and in the trapped sickled erythrocytes in the capillary thrombi of kidneys. The second case probably had the onset of acute sicklemia, developed at least 15 hours before death, when the patient was acutely ill with much vomitus and unobtainable pulse and blood pressure.

There are several interesting points pathologically. First, it is noted that the sickling was more prominent in the infarcted area or the adjacent tissue. This is in accord with the opinion of Diggs and Vorder Bruegge,[14] who stated: "This hemoglobin is liable to become crystalized being long pointed and rigid followed by increase of viscosity and the flow of blood impeded. The hypoxia by the uptake of living blood and tissue cells in areas in which there is stasis tends to exaggerate the sickling, producing a vicious cycle." They also mentioned in unpublished data that the patient with sickle-cell trait may die suddenly when subjected to conditions associated with hypoxia and vascular stasis. Second, the infarcts in the liver and spleen in both cases, especially in the former, showed different ages; some, less acute, with neutrophilic infiltration, and others, more acute, with no inflammatory reaction at all. It is then reasonable to postulate that the initial infarcts caused more hypoxia of the adjacent tissue, in which further sickling of red blood cells results, followed by infarcts of more acute stage. Third, the theory of hypoxia is further strengthened by the finding of bilateral small cystic infarcts in the globus pallidus, which, according to Freeman,[15] occurs in severe tissue hypoxia in cases of carbon monoxide poisoning.

The unanswered question in these two cases is what causes the initial sicklemia. In the second case, moderately severe bronchopneumonia was present, which together with severe generalized arteriosclerosis might be responsible, but there was no anatomical explanation for the first case at all. This brings out the purpose of this paper: that the possiblity of acute sicklemia with fatal multiple infarcts should be kept in mind when aged Negro patients with sickle-cell trait or sickle-cell hemoglobin C disease are admitted with any condition associated with hypoxia or in a shock condition. A fatal outcome can be avoided when routine sickling preparations are carried out on such patients.

Summary

Two fatal cases with infarcts of multiple organs due to acute sicklemia without sickle-cell anemia are reported with pathological findings.

It is pointed out that acute sicklemia with fatal outcome may occur in aged Negro patients who have sickle-cell trait or sickle-cell hemoglobin C disease when there is any condition associated with slight or moderate hypoxia.

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Obituaries

NATHAN CHANDLER FOOT, M.D.

1881-1958

Dr. Nathan Chandler Foot, Professor Emeritus of Surgical Pathology, Cornell University Medical College, and Consultant in Surgical Pathology, The New York Hospital, died Thursday, Sept. 4, 1958, of pulmonary embolism. He is survived by his wife, whom he married in 1910; a daughter, Mrs. John C. Besson; a sister, Valeria D. Foot, and four grandchildren. Another daughter, Dr. Ellen B. Foot Neumann, died in 1945.

NATHAN CHANDLER FOOT, M.D.
1891-1958

Chandler Foot was born in New York City on July 27, 1881. He received his A.B. from Harvard College, in 1903, and his M.D. from the College of Physicians and Surgeons, Columbia University, in 1907, following which he had resident training at The New York Hospital and then spent two years in Germany studying Pathology. He was on the staff of the Department of Pathology at Harvard from 1912 to 1915 and subsequently was on the faculty of the University of Cincinnati College of Medicine, becoming Professor of Pathology at that institution in 1928. In 1932 he came with Dr. George Heuer to the newly created New

York Hospital-Cornell Medical Center to assume the dual post of Professor of Pathology in Surgery and Head of the Laboratory of Surgical Pathology. In this capacity he served with great distinction for sixteen years. In 1948, he became Professor Emeritus; thereafter until his death he engaged in cancer research in the Papanicolaou Cytology Laboratory of Cornell University Medical College, successively in the Departments of Anatomy and Pathology.

He was a member of numerous medical societies, including The American Association of Pathologists and Bacteriologists, of which he was President in 1936; the Harvey Society, and The American Association for the Advancement of Science. During the period 1946-1951, he served as President of the American Board of Pathology; his insistence upon high standards in this formative period has proved of enduring value to the Board. He was also a consultant to the Armed Forces Institute of Pathology, in Washington; Fellow of the College of American Pathologists; a member of the New York Pathological Society, of which he was President in 1936-1937, and a member of the New York Practitioners' Society. His many scientific papers dealt mainly with the histopathologic diagnosis of cancer, and so, too, did his textbooks—"Pathology in Surgery" and "Identification of Tumors"; an ingenious and expert use of special stains, notably those employing silver, contributed largely to his competence in this field.

Chandler Foot was loved and esteemed by his associates and by the long succession of medical students who came into his classes—as much because of his charm and wit and varied interests as for his wide learning and compassion. His geniality made it easy for him to pass on to others the things he knew. In music he was not only an eager and appreciative listener and student—he took his own scores to concerts and followed these closely—but also an accomplished piano and accordian player; many a student-faculty gathering, which might otherwise have been formal and stiff, was made warm and lively by his playing and presence. Admiring art in all its forms, he painted as well, first in oils and later in water colors, particularly ocean and beach scenes; numbers of his works won the critical acclaim of experts, and not a few of them now adorn the homes and offices of his friends. His proficiency in cowboy pool, together with his large measure of what the Scots call innerliness, endeared him to such of his friends as also found this game interesting and relaxing, and with these—artists, writers, and architects, as well as lawyers and physicians—he spent many hours in the clubhouse of The Century Association. A bird-watcher, Chandler Foot knew well the forms, colors, songs, and habits of those creatures and the outlook and language of those of his companions who shared this interest with him. Woodworking and metalworking also gave him pleasure, a beautifully mitered joint no less than the finished product; an elaborate miniature railway station, replete with furnishings, ticket offices, and a motor-driven elevator with stops on three levels, which he designed and made to complement the electric trains of his grandson, provides an example of his work. So, too, with foreign languages, he was not content to be a mere learner: in addition to reading widely in French, German, and Italian literature, he spoke Hungarian fluently and also had knowledge of Chinese; he once posted a directive on the door of the refrigerator in his laboratory telling orderlies and nurses aides in seven languages where to place specimens!

The content of his scientific papers and books, the wealth of carefully studied material which he left for his successors in the Laboratory of Surgical Pathology at The New York Hospital-Cornell Medical Center, and the memories and notes

of the thousands of medical students whom he taught, provide of course the tangible measure of Chandler Foot's achievements as physician, teacher, and scientist. But just as surely it can be said that his most enduring influence as a teacher was exerted upon those of George Heuer's Resident Surgeons who were fortunate enough to have periods of training in his laboratory during the years 1932-1948. Few of these outstanding men, now mature surgeons, failed to respond to his warmth, wit, and enthusiasm, or to respect and learn from his careful clinical judgements, and many would say with gratitude that his guidance largely determined their outlook upon Medicine and life. How fortunate he was to have found wide scope for his talents, and happiness besides! How fortunate, too, are those of us who knew him; for we can recall with pleasure what he was and what he did, and what is more can note with admiration the reflections of his influence in the lives of those who share our memories of him.

JOHN G. KIDD, M.D.

News and Comment

DEATHS

Dr. Joseph Aronson Dies.—Dr. Joseph Aronson, of Philadelphia, died on Oct. 18, 1958. At the time of his death he was making a study of leprosy in Paramaribo, Surinam.

PERSONAL

Dr. Ralph D. Lillie Receives Award.—Dr. Ralph D. Lillie, of the National Institutes of Health, received the Sustaining Membership Award of 1958 of the Association of Military Surgeons in the United States. This award was made in recognition of his many contributions to medicine over a period of almost four decades.

Dr. Albert H. Coons Receives Award.—Dr. Albert H. Coons received the eighth annual Kimble Methodology Research Award at the Conference of State and Provincial Public Health Laboratory Directors, in St. Louis, in 1958, in recognition of his contributions to the field of immunology.

Dr. Paul Kimmelstiel Lectures.—Dr. Paul Kimmelstiel, Professor of Pathology, Marquette University School of Medicine, gave the First Charles Warrington Earle Lecture of the Institute of Medicine of Chicago on Oct. 27. The title of his lecture was "Functional-Morphologic Concept of Nephrosis."

Dr. Thomas B. Magath Receives Award.—Dr. Thomas B. Magath, Mayo Clinic, Rochester, Minn., received the Ward Burdick Award of the American Society of Clinical Pathologists for the year 1958.

Dr. Eleanor M. Humphreys Retires.—Dr. Eleanor M. Humphreys retired on Oct. 1 from the Department of Pathology, The University of Chicago. She served in this department over 35 years and for the past 15 years was head of the Surgical Pathology Laboratory.

ANNOUNCEMENTS

Symposium on Biochemical Cytology of Liver.—The Histochemical Society will present a symposium on Biochemical Cytology of Liver, under the presidency of Dr. Alex B. Novikoff, of the Albert Einstein College of Medicine of Yeshiva University, in Convention Hall, Atlantic City, N. J., April 13, 1959, from 9 a. m. to 12:45 p. m.:

9:00 a. m. A. Ficq (Universite Libre de Bruxelles)
Radioautographic Studies on Nuclear Activity in the Liver

9:40 a. m. T. Peters Jr. (The Mary Imogene Bassett Hospital)
Cytoplasmic Particles and Serum Albumin Synthesis

10:20 a. m. K. R. Porter (The Rockefeller Institute for Medical Research)
Pathological Changes in the Fine Structure of Liver Cells Arising from Azo Dye Feeding

11:00 a. m. Intermission

11:10 a. m. L. L. Miller (University of Rochester School of Medicine and Dentistry)
Physiological Considerations and Liver Cell Function

11:18 a. m. R. E. Shank (Washington University School of Medicine)
Cell Heterogeneity Within the Hepatic Lobule (Quantitative Histochemistry)

11:26 a. m. A. B. Novikoff (Albert Einstein College of Medicine of Yeshiva University)
Cell Heterogeneity Within the Hepatic Lobule (Staining Reactions)

11:34 a. m. M. Simpson (Yale University)
Protein Synthesis in Mitochondria

11:42 a. m. K. Paigen (Roswell Park Memorial Institute)
Genetic Influences on Enzyme Localization (Lysosomes)

11:50 a. m. I. M. Arias (Albert Einstein College of Medicine of Yeshiva University)
A Defect in Microsomal Function in Nonhemolytic Acholuric Jaundice

11:58 a. m. E. N. Leduc (Brown University)
Cell Modulation in Liver Pathology
12:06 p. m. General Discussion

Research Fellowship in Gynecologic Pathology.—The Baptist Memorial Hospital, Jacksonville, Fla., will offer a research fellowship in the general area of research in gynecologic pathology, to begin in the summer of 1959. The hospital currently operates 272 beds. It was opened in September, 1955, in a new air-conditioned building directly on the St. Johns River. The laboratories are attractively located on the second floor, with separate laboratories for histology, bacteriology, hematology, clinical microscopy, and chemistry. A new research laboratory with animal facilities is located on the sixth floor. The hospital is approved for residency training in pathologic anatomy and clinical pathology.

Requirements.—Graduation from an approved medical school, one year of approved internship, and at least one year of approved residency training in pathology or in obstetrics and gynecology.

Stipend.—A stipend of $3,800 per year, with an additional $350 per year each for one or two dependents. No maintenance is furnished. Uniforms are not furnished but are laundered without charge.

Research Program.—An active research program, principally in gynecological pathology, is in progress, supported by grant funds. Four research technicians are currently employed. Present projects include histochemical and cytophysical studies of intraepithelial cervical carcinoma and allied lesions, experimental melanoma, experimental production of ovarian tumors, and histochemical studies in pulmonary fibrosis. A new program of correlation of vaginal cytology with histochemical patterns in ovaries with special reference to aging is in progress. A full battery of histochemical procedures relative to these studies is in being, and interference contrast microscopy is employed where useful.

Duties of the Fellowship.—Major attention will be given to supervised research in the project of aging changes in the ovary. Correlation of clinical setting with research findings in tissues and smears will be stressed. The details of duties will depend in part on the previous training and interests of the fellow.

Opportunities.—An aspirant pathologist should receive carefully supervised research experience in histochemical and allied methods, research planning, and the conduct of research. Opportunity for individual investigation will be made available to suitable persons. An aspirant obstetrician-gynecologist should receive similar experience, with basic training in gynecologic pathology and in the application of research to patient problems.

Supervision.—The program is under the supervision of Dr. Alvan G. Foraker, Pathologist, former Associate Professor of Pathology at Emory University School of Medicine and at the University of Texas Post Graduate School of Medicine. Dr. Sam W. Denham, Attending Obstetrician-Gynecologist, is clinical co-investigator in gynecologic research.

Applications or inquiries should be addressed to Alvan G. Foraker, M.D., Pathologist, Baptist Memorial Hospital, 800 Miami Rd., Jacksonville (7), Fla.

Films.—The following films should be of interest to pathologists.

Title: Dye Transfer by Renal Tubules
(9 minutes, sound, color, 1958)
Authors: E. L. Becker, M.D., and Sidney Solomon, Ph.D.
Producer: Department of Visual Education, Medical College of Virginia, Richmond, Va.
Sponsor: American Medical Association
Distribution: Department of Visual Education, Medical College of Virginia.

Title: Respiratory Resuscitation Techniques
(44 minutes, sound, color, 1958)
Author: Peter Safar, M.D.
Scientific Advisers: J. O. Elam, M.D., and D. G. Greene, M.D.
Producer: Walter Reed Army Institute of Research
Sponsors: Department of the Army and Burroughs Wellcome & Company, Inc.
Distribution: Medical Audio-Visual Division, Walter Reed Army Institute of Research, Walter Reed Army Medical Center, Washington, D. C.

Title: Machine Mimics Man: The Artificial Kidney
(24 minutes, sound, color, 1958)

Authors: Willem J. Kolff, M.D.; W. A. Kelemen, M.D.; George Schreiner, M.D., and L. B. Berman, M.D.
Producer: Mervin LaRue, Inc., Chicago
Sponsor and Distributor: Travenol Laboratories, Inc., Morton Grove, Ill.

Title: Dynamics of Phagocytosis
(28 minutes, sound, black and white, 1958)
Author: Armine T. Wilson, M.D., A. I. DuPont Institute, Wilmington, Del.
Producer: Leo L. Leveridge, M.D., Pfizer Laboratories, Division of Chas. Pfizer & Company
Sponsor: Pfizer Laboratories
Distribution: Pfizer Laboratories Professional Service Representatives

Title: The Mitral Valve—Pathology and Surgery
(19 minutes, sound, color, 1957)
Authors: R. P. Glover, M.D.; M. C. Davila, M.D., and R. G. Tront, M.D.
Producers: J. J. Magee and J. E. Sumner
Sponsor: Cardiovascular Research Foundation, Presbyterian Hospital, Philadelphia; U. S. Public Health Service and American Heart Association
Distribution: E. R. Squibb & Sons Film Library, 745 5th Ave., New York (22)

Title: The Human Cell and the Cytotechnologist
(23 minutes, sound, color, 1957)
Producer: Churchill-Wexler Productions
Sponsors: National Cancer Institute and American Cancer Society, with College of American Pathologists, American Society of Clinical Pathologists, and American Society of Medical Technologists
Distribution: National Committee for Careers in Medical Technology, 1785 Massachusetts Ave., N. W., Washington (6) D. C.

International Scientific Meeting of International Academy of Pathology.—The International Academy of Pathology will hold an international scientific meeting, in London, on June 20, 21, 22, and 23, 1960, at the Royal College of Surgeons. There will be a one-day course on the kidney, a slide seminar on the breast, open sessions for presentation of general scientific papers by members, and scientific exhibits. Dr. Lauren V. Ackerman, of St. Louis; Prof. R. W. Scarff, of London, and Prof. Charles Oberling, of Paris, will be the moderators for the slide seminar. The last half-day program will be a joint meeting with the pathologists of the American Goiter Association. There will be a symposium on cancer of the thyroid and another on thyroiditis. Dr. George J. Cunningham will be the secretary of the London meeting. Further details of the meeting will be published subsequently.

Books

The Medical World of the Eighteenth Century. By Lester S. King, M.D. Price, $5.75. Pp. 346. The University of Chicago Press, 58th St. & Ellis Ave., Chicago 37, 1958.

This book is a resultant of the philosophical and historical interests of a pathologist who believes that an understanding of today's medical structure requires an appreciation of its historical development. In writing the book, the author thought of the eighteenth century as representing, "perhaps, the adolescence of present-day medicine." As Dr. Ilza Veith says in the "Foreword": "This is not a medical history of the century; it is, instead, a series of loosely related essays dealing with phases of the medical scene that are generally neglected in the formal histories. As such it presents to us a more vivid and graphic account of actual medical life than we are accustomed to reading." Dr. King likens himself to a photographer who seeks to capture certain scenes of the medical past by pointing "his camera backward" in an attempt to "capture certain facets which appear to me both interesting and significant." This he has done, and he has done it admirably.

In the ten chapters he deals concisely and interestingly with such matters as "Apothecary and Physician," "Quack and Empiric," "Nosology," "Of Fevers," "The Development of Medical Ethics," "The Rise of Modern Pathology," and "The Practice of Medicine." The two chapters dealing with Boerhaave as a systematist and as a scientist are meant to portray the attributes of probably the most influential physician of the eighteenth century. The chapter entitled "Similia Similibus" is a fascinating account of Samuel Hahnemann.

In the writing of the book, Dr. King has attempted to maintain objectivity, and in looking backward, he has preferred "to note similarities rather than differences." By this approach he finds eighteenth-century doctors as clever as their twentieth-century successors, "just as perceptive and clear-thinking, exerting powers of observation and inference just as keen." They were also "as muddleheaded, obtuse, grasping, prejudiced, and contentious as their descendants." In any case, they were living in times in which the foundations of modern medicine were being laid.

Both the author and The University of Chicago Press are to be commended for the production of such an excellent book, with its enlightening picture of the beginnings of scientific medicine. It should be of interest to pathologists, to physicians and medical students, and, indeed, to anyone interested in the historical development of medicine.

Periodontics: A Concept—Theory and Practice. Edited by Balint Orban, M.D., D.D.S., with co-editors Frank M. Wentz, M.S., Ph.D., D.D.S.; Frank G. Everett, M.S., D.M.D., M.D., and Daniel A Grant, D.D.S. Price, $14.50. Pp. 537, with 439 illustrations. The C. V. Mosby Company, 3207 Washington Blvd., St. Louis 3, 1958.

This book, as the title indicates, is very comprehensive in its consideration of the practice of periodontics. It is profusely illustrated with the histological, roentgenographic, and clinical appearance of the normal, as well as the types of gingivitis and pericdontitis encountered in clinical practice. Diagnostic features, treatment, and postoperative sequelae are dealt with, including an excellent chapter on traumatic periodontal disturbances, with consideration of the treatment of occlusal disharmonies. The text is easy reading, attractive in its manner of presentation by the use of actual cases, and also has an excellent bibliography. One criticism of this book is its lack of consideration of all the systemic conditions which might cause gingival hyperplasia, one of them being the leukemias. Aside from this small criticism, which the author can correct in future editions, this textbook can be highly recommended to the student of periodontology.

Lehrbuch der speziellen pathologischen Anatomie. III Band, 3 Lieferung. By Dr. Eduard Kaufmann; edited by Prof. Dr. Martin Staemmler. Price, DM31. Pp. 155, with 104 illustrations. Walter de Gruyter, Genthiner Strasse 13, Berlin W. 35, 1958.

This part of the "new Kaufmann" constitutes the third section of the third and last volume of this new work. It comprises a treatise on disturbances of circulation and disease of the central nervous system, including cerebral edema and cerebral swelling, by M. Staemmler, and another, on malformations of the central nervous system, by G. Peters and O. E. Lund.

Besides the chapters on organic blood vessel diseases, there is one on "hypertonic" blood vessel disease. This, the author justifies, was done because of the importance of arterial hypertension in massive cerebral hemorrhage. According to his experience, there is no relation between hypertension and generalized arteriosclerosis. The characteristic changes are the so-called hyalinosis of small intracerebral arteries and not only of the arterioles, with narrowing of their lumens, and also arterio- and arteriolonecrosis. The former change is responsible for the development of small foci of encephalomalacia, while the latter cause the large cerebral hemorrhages. Study of cerebral malformations is prefaced by a discussion of their pathogenesis, of the normal development of brain and spinal cord, and of the microscopic differentiation of cortex and white substance of the brain. A number of anomalies usually mentioned only in passing are given in detail, such as changes occurring in Mongolism and heterotopias, i. e., the findings of islands of cortical tissue in abnormal locations.

This section of the third volume, as the other parts of the "new Kaufmann" is a valuable addition to the relatively meager descriptions found in ordinary texts. While the medical student might be confused by the wealth of material and detailed analysis of pathologic entities, it contains important information for the specialist who needs it.

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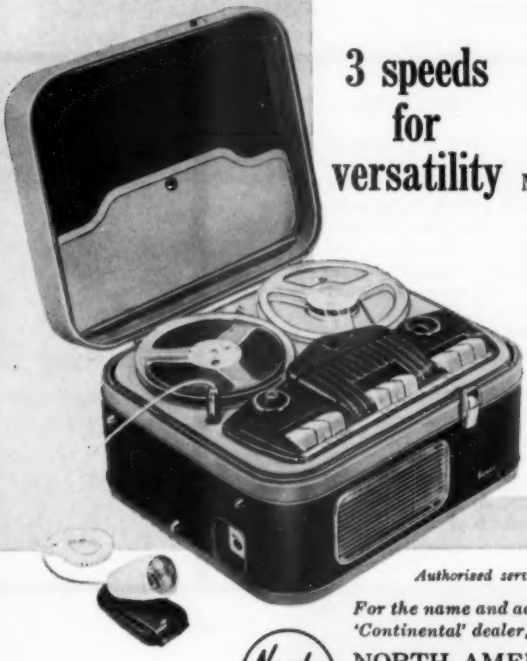

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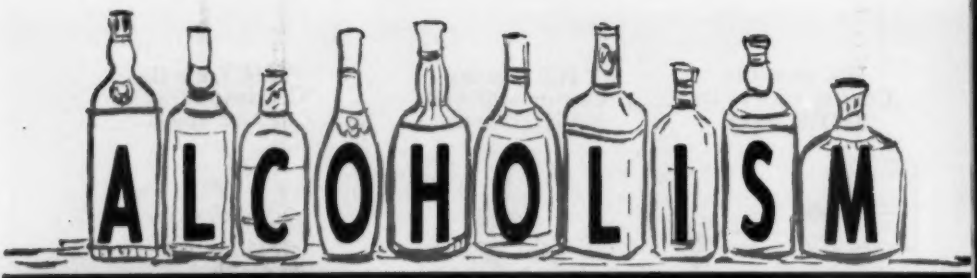

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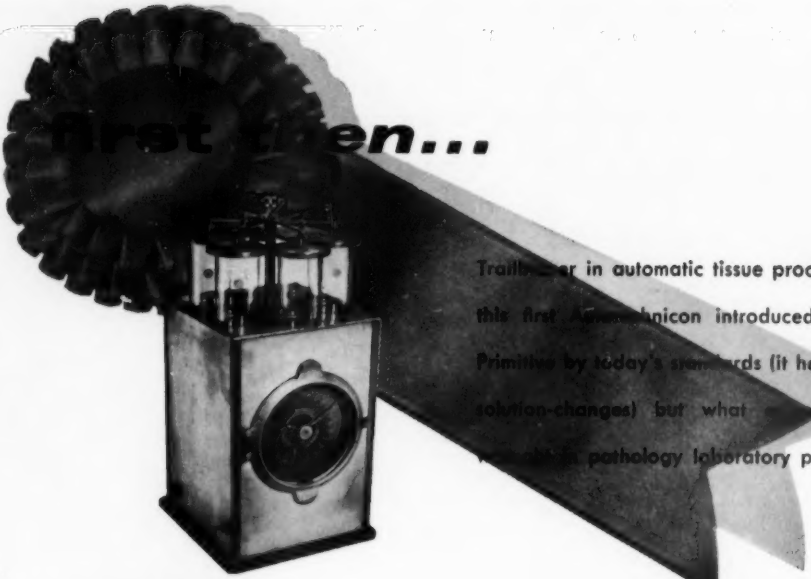

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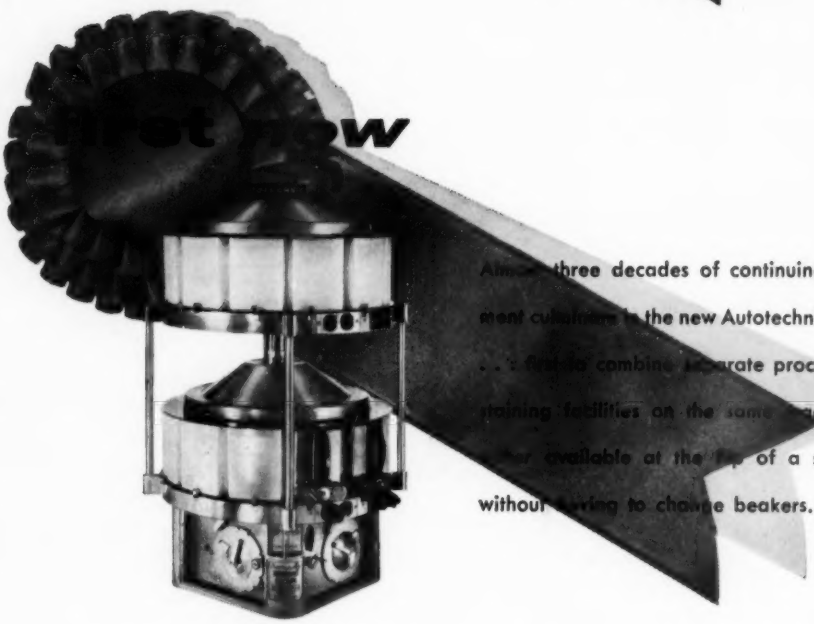

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